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(71) Applicant *(for all designated States except US)*: **BAYER AKTIENGESELLSCHAFT** [DE/DE]; 51368 Leverkusen (DE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **ZHU, Zhimin** [CN/US]; 45 Hinckley Road, Waban, MA 02468 (US).

(74) Common Representative: **BAYER AKTIENGESELLSCHAFT**; 51368 Leverkusen (DE).

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(54) Title: REGULATION OF HUMAN CITRON RHO/RAC-INTERACTING KINASE

(57) Abstract: Reagents that regulate human CRIK and reagents which bind to human CRIK gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, obesity, a CNS disorder or COPD.

REGULATION OF HUMAN CITRON RHO/RAC-INTERACTING KINASE

This application incorporates by reference and claims the benefit of co-pending provisional applications Serial No. 60/301,841 filed July 2, 2001, Serial No. 60/338,651 filed December 11, 2001 and Serial No. 60/375,014 filed April 25, 2002

TECHNICAL FIELD OF THE INVENTION

The invention relates to the regulation of human citron rho/rac-interacting kinase (CRIK).

BACKGROUND OF THE INVENTION

Kinases are involved in a variety of disease processes. There is a need in the art to identify related enzymes, which can be regulated for therapeutic effects.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a human CRIK. This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a human citron rho/rac-interacting kinase polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 97% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a human citron rho/rac-interacting kinase polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 97% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

Binding between the test compound and the human citron rho/rac-interacting kinase polypeptide is detected. A test compound which binds to the human citron rho/rac-interacting kinase polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the activity of the human citron rho/rac-interacting kinase.

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a human citron rho/rac-interacting kinase polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 24; and

the nucleotide sequence shown in SEQ ID NO: 24.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the human citron rho/rac-interacting kinase through interacting with the human citron rho/rac-interacting kinase mRNA.

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a human citron rho/rac-interacting kinase polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 97% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

A human citron rho/rac-interacting kinase activity of the polypeptide is detected. A test compound which increases human citron rho/rac-interacting kinase activity of the polypeptide relative to human citron rho/rac-interacting kinase activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases human citron rho/rac-interacting kinase activity of the polypeptide relative to human citron rho/rac-interacting kinase activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a human citron rho/rac-interacting kinase product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 24; and

the nucleotide sequence shown in SEQ ID NO: 24.

Binding of the test compound to the human citron rho/rac-interacting kinase product is detected. A test compound which binds to the human citron rho/rac-interacting kinase product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a human citron rho/rac-interacting kinase polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 24; and

the nucleotide sequence shown in SEQ ID NO: 24.

Human citron rho/rac-interacting kinase activity in the cell is thereby decreased.

The invention thus provides a human CRIK that can be used to identify test compounds that may act, for example, as activators or inhibitors at the enzyme's active site. Human CRIK and fragments thereof also are useful in raising specific antibodies that can block the enzyme and effectively reduce its activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the DNA-sequence encoding a citron rho/rac-interacting kinase Polypeptide (SEQ ID NO:1).

Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig.1 (SEQ ID NO:2).

Fig. 3 shows the amino acid sequence of the protein identified by trembl|AF086824|AF086824_1 (SEQ ID NO:3).

Fig. 4 shows the DNA-sequence encoding a citron rho/rac-interacting kinase Polypeptide (SEQ ID NO:4).

Fig. 5 shows the amino acid sequence of the protein identified by swiss|O14578|CTRO_HUMAN (SEQ ID NO:5).

Fig. 6 shows the DNA-sequence of a protein identified by trembl|AB023166|AB023166_1 (SEQ ID NO:6).

Fig. 7 shows the amino acid sequence of the protein identified by swissnew|P54265|DMK_MOUSE (SEQ ID NO:7).

Fig. 8 shows the BLASTP - alignment of 543_Protein (SEQ ID NO:2) against trembl|AF086824|AF086824_1 (SEQ ID NO:3).

Fig. 9 shows the BLASTP - alignment of 543_Protein (SEQ ID NO:2) against swiss|O14578|CTRO_HUMAN (SEQ ID NO:5).

Fig. 10 shows the BLASTP - alignment of 543_Protein (SEQ ID NO:2) against aageneseq|AAB43359|AAB43359.

Fig. 11 shows the BLASTP - alignment of 543_Protein (SEQ ID NO:2) against trembl|AB023166|AB023166_1 (SEQ ID NO:6).

Fig. 12 shows the BLASTP - alignment of 543_Protein (SEQ ID NO:2) against swissnew|P54265|DMK_MOUSE (SEQ ID NO:7).

Fig. 13 shows the BLASTP - alignment of 543_Protein (SEQ ID NO:2) against pdb|1CDK|1CDK-A.

Fig. 14 shows the HMMPFAM - alignment of 543_Protein (SEQ ID NO:2) against pfam|hmm|pkinase.

Fig. 15 shows the HMMPFAM - alignment of 543_Protein (SEQ ID NO:2) against pfam|hmm|PH.

Fig. 16 shows the HMMPFAM - alignment of 543_Protein (SEQ ID NO:2) against pfam|hmm|CNH.

Fig. 17 shows the HMMPFAM - alignment of 543_Protein (SEQ ID NO:2) against pfam|hmm|DAG_PE-bind.

Fig. 18 shows the HMMPFAM - alignment of 543_Protein (SEQ ID NO:2) against pfam|hmm|pkinase_C.

Fig. 19 shows the Prosite search results.

Fig. 20 shows the Genewise output.

Fig. 21 shows the Relative expression of human citron rho/rac-interacting kinase.

Fig. 22 shows the TBLASTN – alignment of 543_Protein against emnew|AX166510|AX166510 Sequence 1 from Patent WO0138503.//:gbnew|AX166510|AX166510 Sequence Patent WO0138503.

Fig. 23 shows the TBLASTN – alignment of 543_Protein against BAYER_LIB_DNA|wu_37300600 Bayer Corp Pharma Proprietary OP Library: Fat Rat Hypothalamus Linda Oct 15 15:45:51 EDT 1999

Fig. 24 shows the DNA-sequence encoding a citron rho/rac-interacting kinase Polypeptide.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide from the group consisting of:

a) a polynucleotide encoding a human citron rho/rac-interacting kinase polypeptide comprising an amino acid sequence selected from the group consisting of:
amino acid sequences which are at least about 97% identical to the amino acid sequence shown in SEQ ID NO: 2; and
the amino acid sequence shown in SEQ ID NO: 2.

b) a polynucleotide comprising the sequence of SEQ ID NOS: 1 or 24;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b) and encodes a human citron rho/rac-interacting kinase polypeptide;

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes a human citron rho/rac-interacting kinase polypeptide; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes a human citron rho/rac-interacting kinase polypeptide.

Furthermore, it has been discovered by the present applicant that a novel CRIK, particularly a human CRIK, can be used in therapeutic methods to treat obesity, a CNS disorder, diabetes or COPD. Human CRIK comprises the amino acid sequence shown in SEQ ID NO:2. A coding sequence for human CRIK is shown in SEQ ID NO:1. This sequence is contained within the longer sequence shown in SEQ ID NO:4, which is located on chromosome 12q24.2. Related ESTs are expressed in bone marrow, denis_drash (pediatric kidney tumors), epithelioid carcinoma

(pancreas), colon_ins (colon cancer cell line), uterus_tumor, glioblastoma with EGFR amplification, colon, nervous, nervous tumor, and bladder_tumor.

Human CRIK is 96% identical over 2056 amino acids to trembl|AF086824|AF086824_1 (SEQ ID NO:3) (FIG. 1), 100% identical over 1286 amino acids to swiss|O14578|CTRO_HUMAN (SEQ ID NO:5) (FIG. 2), 100% identical over 1286 amino acids to SEQ ID NO:6246 of aageneseq|AAB43359|AAB43359 (FIG. 3), 100% over 940 amino acids to trembl|AB023166|AB023166_1 (SEQ ID NO:6) (FIG. 4), and 38% identical over 522 amino acids to swissnew|P54265|DMK_MOUSE (SEQ ID NO:7) (FIG. 5).

Human CRIK of the invention is expected to be useful for the same purposes as previously identified CRIK enzymes. Human CRIK is believed to be useful in therapeutic methods to treat disorders such as CNS disorders, obesity,y and COPD. Human CRIK also can be used to screen for human CRIK activators and inhibitors.

Polypeptides

Human CRIK polypeptides according to the invention comprise at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975, 1000, 1025, 1050, 1075, 1100, 1125, 1150, 1175, 1200, 1225, 1250, 1275, 1300, 1325, 1350, 1375, 1400, 1425, 1450, 1475, 1500, 1525, 1550, 1575, 1600, 1625, 1650, 1675, 1700, 1725, 1750, 1775, 1800, 1825, 1850, 1875, 1900, 1925, 1950, 1975, 2000, 2025, 2050, or 2054 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:2 or a biologically active variant thereof, as defined below. A CRIK polypeptide of the invention therefore can be a portion of a CRIK protein, a full-length CRIK protein, or a fusion protein comprising all or a portion of a CRIK protein.

Biologically Active Variants

Human CRIK polypeptide variants which are biologically active, *e.g.*, retain enzymatic activity, also are human CRIK polypeptides. Preferably, naturally or non-naturally occurring human CRIK polypeptide variants have amino acid sequences which are at least about 97, 98, of 99% identical to the amino acid sequence shown in SEQ ID NO:2 or a fragment thereof. Percent identity between a putative human CRIK polypeptide variant and an amino acid sequence of SEQ ID NO:2 is determined by conventional methods. See, for example, Altschul *et al.*, *Bull. Math. Bio. 48*:603 (1986), and Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA 89*:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff & Henikoff, 1992.

Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson & Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative variant. The FASTA algorithm is described by Pearson & Lipman, *Proc. Nat'l Acad. Sci. USA 85*:2444(1988), and by Pearson, *Meth. Enzymol. 183*:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.*, SEQ ID NO: 2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence the ktup value), then the trimmed initial regions are examined to determine whether the regions can be

joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch- Sellers algorithm (Needleman & Wunsch, *J. Mol. Biol.48*:444 (1970); Sellers, *SIAM J. Appl. Math.26*:787 (1974)), which allows for amino acid insertions and deletions. Preferred parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol. 183*:63 (1990).

FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as default.

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a human CRIK polypeptide can be found using computer programs well known in the art, such as DNASTAR software.

The invention additionally, encompasses CRIK polypeptides that are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups,

proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications can be carried out by known techniques including, but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.

Additional post-translational modifications encompassed by the invention include, for example, *e.g.*, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. The CRIK polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

The invention also provides chemically modified derivatives of CRIK polypeptides that may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization can be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol, and the like. The polypeptides can be modified at random or predetermined positions within the molecule and can include one, two, three, or more attached chemical moieties.

Whether an amino acid change or a polypeptide modification results in a biologically active CRIK polypeptide can readily be determined by assaying for enzymatic activity, as described for example, in Di Cunto F. *et al.*, J Biol Chem. 1998 Nov 6;273(45):29706-11.

Fusion Proteins

Fusion proteins are useful for generating antibodies against CRIK polypeptide amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins that interact with portions of a CRIK polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A CRIK polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975, 1000, 1025, 1050, 1075, 1100, 1125, 1150, 1175, 1200, 1225, 1250, 1275, 1300, 1325, 1350, 1375, 1400, 1425, 1450, 1475, 1500, 1525, 1550, 1575, 1600, 1625, 1650, 1675, 1700, 1725, 1750, 1775, 1800, 1825, 1850, 1875, 1900, 1925, 1950, 1975, 2000, 2025, 2050, or 2054 contiguous amino acids of SEQ ID NO:2 or of a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length CRIK protein.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β-galactosidase, β-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4

DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the CRIK polypeptide-encoding sequence and the heterologous protein sequence, so that the CRIK polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from SEQ ID NO:1 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human CRIK polypeptide can be obtained using CRIK polypeptide polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of CRIK polypeptide, and expressing the cDNAs as is known in the art.

Polynucleotides

A CRIK polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a CRIK polypeptide. A coding sequence for human CRIK is shown in SEQ ID NO:1.

Degenerate nucleotide sequences encoding human CRIK polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, 98, or 99% identical to the nucleotide sequence shown in SEQ ID NO:1 or its complement also are CRIK polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of CRIK polynucleotides that encode biologically active CRIK polypeptides also are CRIK polynucleotides. Polynucleotide fragments comprising at least 8, 9, 10, 11, 12, 15, 20, or 25 contiguous nucleotides of SEQ ID NO:1 or its complement also are CRIK polynucleotides. These fragments can be used, for example, as hybridization probes or as antisense oligonucleotides.

Identification of Polynucleotide Variants and Homologs

Variants and homologs of the CRIK polynucleotides described above also are CRIK polynucleotides. Typically, homologous CRIK polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known CRIK polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50 °C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30%

basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the CRIK polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of CRIK polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5 °C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol. 81*, 123 (1973). Variants of human CRIK polynucleotides or CRIK polynucleotides of other species can therefore be identified by hybridizing a putative homologous CRIK polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO:1 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to CRIK polynucleotides or their complements following stringent hybridization and/or wash conditions also are CRIK polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20 °C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a CRIK polynucleotide having a nucleotide sequence shown in SEQ ID NO:1 or the complement thereof and a polynucleotide sequence which is at least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences

can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A. 48*, 1390 (1962):

$T_m = 81.5\ ^\circ C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%formamide) - 600/l$,
where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65 °C, or 50% formamide, 4X SSC at 42 °C, or 0.5X SSC, 0.1% SDS at 65 °C. Highly stringent wash conditions include, for example, 0.2X SSC at 65 °C.

Preparation of Polynucleotides

A CRIK polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated CRIK polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments, which comprise CRIK nucleotide sequences. Isolated polynucleotides are in preparations that are free or at least 70, 80, or 90% free of other molecules.

Human CRIK cDNA molecules can be made with standard molecular biology techniques, using CRIK mRNA as a template. Human CRIK cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize CRIK polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a CRIK polypeptide having, for example, an amino acid sequence shown in SEQ ID NO:2 or a biologically active variant thereof.

Extending Polynucleotides

Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2,* 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res. 16*, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72 °C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119,

1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) that are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.* GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA that might be present in limited amounts in a particular sample.

Obtaining Polypeptides

Human CRIK polypeptides can be obtained, for example, by purification from human cells, by expression of CRIK polynucleotides, or by direct chemical synthesis.

Protein Purification

Human CRIK polypeptides can be purified from any cell that expresses the polypeptide, including host cells that have been transfected with CRIK expression constructs. A purified CRIK polypeptide is separated from other compounds that normally associate with the CRIK polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. A preparation of purified CRIK polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

Expression of Polynucleotides

To express a CRIK polynucleotide, the polynucleotide can be inserted into an expression vector that contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods that are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding CRIK polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a CRIK polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a CRIK polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the CRIK polypeptide. For example, when a large quantity of a CRIK polypeptide is needed for the induction of antibodies, vectors which direct

high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the CRIK polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem. 264*, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding CRIK polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J. 6*, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*, 1671-1680, 1984; Broglie *et al.*, *Science 224*, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such

techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express a CRIK polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding CRIK polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of CRIK polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda cells* or *Trichoplusia* larvae in which CRIK polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 3224-3227, 1994).

Mammalian Expression Systems

A number of viral-based expression systems can be used to express CRIK polypeptides in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding CRIK polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus that is capable of expressing a CRIK polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. 81*, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding CRIK polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a CRIK polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ. 20*, 125-162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed CRIK polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells that have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express CRIK polypeptides can be

transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced CRIK sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines.

These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*, 817-23, 1980) genes which can be employed in *tk*$^-$ or *aprt*$^-$ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. 77*, 3567-70, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol. 150*, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85*, 8047-51, 1988). Visible markers such as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol. 55*, 121-131, 1995).

Detecting Expression

Although the presence of marker gene expression suggests that the CRIK polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a CRIK polypeptide is inserted within a marker gene sequence, transformed cells containing sequences that encode a CRIK polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a CRIK polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the CRIK polynucleotide.

Alternatively, host cells which contain a CRIK polynucleotide and which express a CRIK polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques that include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a CRIK polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a CRIK polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a CRIK polypeptide to detect transformants that contain a CRIK polynucleotide.

A variety of protocols for detecting and measuring the expression of a CRIK polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a CRIK polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et*

al., SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med. 158*, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding CRIK polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a CRIK polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding a CRIK polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode CRIK polypeptides can be designed to contain signal sequences which direct secretion of soluble CRIK polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound CRIK polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a CRIK polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the CRIK polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a CRIK polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif. 3*, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the CRIK polypeptide from the fusion protein. Vectors that contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441-453, 1993.

Chemical Synthesis

Sequences encoding a CRIK polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a CRIK polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of CRIK polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic CRIK polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the CRIK polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce CRIK polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life that is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter CRIK polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a CRIK polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv, which are capable of binding an epitope of a CRIK polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of a CRIK polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody that specifically binds to the immunogen.

Typically, an antibody which specifically binds to a CRIK polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to CRIK polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a CRIK polypeptide from solution.

Human CRIK polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a CRIK polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response.

Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies that specifically bind to a CRIK polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature 256*, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 6851-6855, 1984; Neuberger *et al.*, *Nature 312*, 604-608, 1984; Takeda *et al.*, *Nature 314*, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies that specifically bind to a CRIK polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies that specifically bind to CRIK polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91).

Antibodies which specifically bind to CRIK polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833-3837, 1989; Winter *et al.*, *Nature 349*, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in

WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a CRIK polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences that are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of CRIK gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol. Biol. 20*, 1-8, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev. 90*, 543-583, 1990.

Modifications of CRIK gene expression can be obtained by designing antisense oligonucleotides that will form duplexes to the control, 5', or regulatory regions of the CRIK gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a CRIK polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a CRIK polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent CRIK nucleotides, can provide sufficient targeting specificity for CRIK mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular CRIK polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a CRIK polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying

numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal *et al.*, *Trends Biotechnol. 10*, 152-158, 1992; Uhlmann *et al.*, *Chem. Rev. 90*, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett. 215*, 3539-3542, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.*, Cech, *Science 236*, 1532-1539; 1987; Cech, *Ann. Rev. Biochem. 59*, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol. 2*, 605-609; 1992, Couture & Stinchcomb, *Trends Genet. 12*, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.*, Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a CRIK polynucleotide can be used to generate ribozymes that will specifically bind to mRNA transcribed from the CRIK polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see* Haseloff *et al. Nature 334*, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a CRIK RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate CRIK RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease CRIK expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors that induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Differentially Expressed Genes

Described herein are methods for the identification of genes whose products interact with human CRIK. Such genes may represent genes that are differentially expressed in disorders including, but not limited to, obesity, CNS disorders, and COPD. Further, such genes may represent genes that are differentially regulated in response to manipulations relevant to the progression or treatment of such diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human CRIK gene or gene product may itself be tested for differential expression.

The degree to which expression differs in a normal versus a diseased state need only be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quantitative RT (reverse transcriptase), PCR, and Northern analysis.

Identification of Differentially Expressed Genes

To identify differentially expressed genes total RNA or, preferably, mRNA is isolated from tissues of interest. For example, RNA samples are obtained from tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique that does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel *et al.*, ed., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York, 1987-1993. Large numbers of tissue samples may readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

Transcripts within the collected RNA samples that represent RNA produced by differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 85*, 208-12, 1988), subtractive hybridization (Hedrick *et al.*, *Nature 308*, 149-53; Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 2825, 1984), and, preferably, differential display (Liang & Pardee, *Science 257*, 967-71, 1992; U.S. Patent 5,262,311).

The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human CRIK. For example, treatment may include a modulation of expression of the differentially expressed genes and/or the gene encoding the human CRIK. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human CRIK gene or gene product are up-regulated or down-regulated.

Screening Methods

The invention provides assays for screening test compounds that bind to or modulate the activity of a CRIK polypeptide or a CRIK polynucleotide. A test compound preferably binds to a CRIK polypeptide or polynucleotide. More preferably, a test compound decreases or increases enzymatic activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced

recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des. 12*, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem. 37*, 2678, 1994; Cho *et al.*, *Science 261*, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al.*, *J. Med. Chem. 37*, 1233, 1994). Libraries of compounds can be presented in solution (*see*, *e.g.*, Houghten, *BioTechniques 13*, 412-421, 1992), or on beads (Lam, *Nature 354*, 82-84, 1991), chips (Fodor, *Nature 364*, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*, 1865-1869, 1992), or phage (Scott & Smith, *Science 249*, 386-390, 1990; Devlin, *Science 249*, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 6378-6382, 1990; Felici, *J. Mol. Biol. 222*, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to CRIK polypeptides or polynucleotides or to affect CRIK activity or CRIK gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50

to 500 μl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 19,* 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity 2,* 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support.

When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule that binds to and occupies, for example, the active site of the CRIK polypeptide, such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules.

In binding assays, either the test compound or the CRIK polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound that is bound to the CRIK polypeptide can then be accomplished, for example, by direct counting of radioemmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a CRIK polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a CRIK polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a CRIK polypeptide (McConnell *et al.*, *Science 257*, 1906-1912, 1992).

Determining the ability of a test compound to bind to a CRIK polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants

(*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a CRIK polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell 72*, 223-232, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 12046-12054, 1993; Bartel *et al.*, *BioTechniques 14*, 920-924, 1993; Iwabuchi *et al.*, *Oncogene 8*, 1693-1696, 1993; and Brent W094/10300), to identify other proteins which bind to or interact with the CRIK polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding a CRIK polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein that interacts with the CRIK polypeptide.

It may be desirable to immobilize either the CRIK polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the CRIK polypeptide (or polynucleotide) or the test compound can be bound

to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the enzyme polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a CRIK polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the CRIK polypeptide is a fusion protein comprising a domain that allows the CRIK polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed CRIK polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a CRIK polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated CRIK polypeptides (or polynucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce

Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a CRIK polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the active site of the CRIK polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the CRIK polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of the CRIK polypeptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a CRIK polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a CRIK polypeptide or polynucleotide can be used in a cell-based assay system. A CRIK polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to a CRIK polypeptide or polynucleotide is determined as described above.

Enzyme Assays

Test compounds can be tested for the ability to increase or decrease the enzymatic activity of a human CRIK polypeptide. Enzymatic activity can be measured, for example, as described in Di Cunto *et al.*, J Biol Chem. 1998 Nov 6;273(45):29706-11.

Enzyme assays can be carried out after contacting either a purified CRIK polypeptide, a cell membrane preparation, or an intact cell with a test compound. A test compound that decreases an enzymatic activity of a CRIK polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as

a potential therapeutic agent for decreasing CRIK activity. A test compound which increases an enzymatic activity of a human CRIK polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for increasing human CRIK activity.

Gene Expression

In another embodiment, test compounds that increase or decrease CRIK gene expression are identified. A CRIK polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the CRIK polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of CRIK mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a CRIK polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a CRIK polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell that expresses a CRIK polynucleotide can be used in a cell-based assay system. The CRIK polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions that can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a CRIK polypeptide, CRIK polynucleotide, ribozymes or antisense oligonucleotides, antibodies which specifically bind to a CRIK polypeptide, or mimetics, activators, or inhibitors of a CRIK polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be

formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxy-propylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as

Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

Human CRIK can be regulated to treat obesity, CNS disorders, and COPD.

Obesity. Obesity and overweight are defined as an excess of body fat relative to lean body mass. An increase in caloric intake or a decrease in energy expenditure or both can bring about this imbalance leading to surplus energy being stored as fat. Obesity is associated with important medical morbidities and an increase in mortality. The causes of obesity are poorly understood and may be due to genetic factors, environmental factors or a combination of the two to cause a positive energy balance. In contrast, anorexia and cachexia are characterized by an imbalance in energy intake versus energy expenditure leading to a negative energy balance and weight loss. Agents that either increase energy expenditure and/or decrease energy intake, absorption or storage would be useful for treating obesity, overweight, and associated comorbidities. Agents that either increase energy intake and/or decrease energy expenditure or increase the amount of lean tissue would be useful for treating cachexia, anorexia and wasting disorders.

This gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating obesity, overweight, anorexia, cachexia, wasting disorders, appetite suppression, appetite enhancement, increases or decreases in satiety, modulation of body weight, and/or other eating disorders such as bulimia. Also this gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating obesity/overweight-associated comorbidities including hypertension, type 2 diabetes, coronary artery disease, hyperlipidemia, stroke, gallbladder disease, gout, osteoarthritis, sleep apnea and respiratory problems, some types of cancer including endometrial, breast, prostate, and colon cancer, thrombolic disease, polycystic ovarian syndrome, reduced fertility, complications of pregnancy, menstrual irregularities, hirsutism, stress incontinence, and depression.

The hypothalamus has been considered as the feeding control center. Many neuropeptides, hormones, neurotransmitters, etc. that play important roles in he control of energy homoeostasis have been identified in the hypothalamus. See *J. Lip. Res. 40,* 1735-46, 1999; *Pharm. Rev. 52*, 35-61, 2000. Leptin signaling pathway, MC4, and 5-HT2C systems in the hypothalamus play critical roles in the control of body weight homeostasis. Therefore, a gene selectively expressed in the hypothalamus, such as the human CRIK of the invention, is a potential obesity target.

CNS disorders. Central and peripheral nervous system disorders also can be treated, such as primary and secondary disorders after brain injury, disorders of mood, anxiety disorders, disorders of thought and volition, disorders of sleep and wakefulness, diseases of the motor unit, such as neurogenic and myopathic disorders, neurodegenerative disorders such as Alzheimer's and Parkinson's disease, and processes of peripheral and chronic pain.PAR.Pain that is associated with CNS disorders also can be treated by regulating the activity of human CRIK. Pain which can be treated includes that associated with central nervous system disorders, such as multiple sclerosis, spinal cord injury, sciatica, failed back surgery syndrome, traumatic brain injury, epilepsy, Parkinson's disease, post-stroke, and vascular lesions in the brain and spinal cord (*e.g.*, infarct, hemorrhage, vascular malformation). Non-central neuropathic pain includes that associated with post mastectomy pain, reflex sympathetic dystrophy (RSD), trigeminal neuralgia-radioculopathy, post-surgical pain, HIV/AIDS related pain, cancer pain, metabolic neuropathies (*e.g.*, diabetic neuropathy, vasculitic neuropathy secondary to connective tissue disease), paraneoplastic polyneuropathy associated, for example, with carcinoma of lung, or leukemia, or lymphoma, or carcinoma of prostate, colon or stomach, trigeminal neuralgia, cranial neuralgias, and post-herpetic neuralgia. Pain associated with cancer and cancer treatment also can be treated, as can headache pain (for example, migraine with aura, migraine without aura, and other migraine disorders), episodic and chronic tension-type headache, tension-type like headache, cluster headache, and chronic paroxysmal hemicrania.

COPD. Chronic obstructive pulmonary (or airways) disease (COPD) is a condition defined physiologically as airflow obstruction that generally results from a mixture of emphysema and peripheral airway obstruction due to chronic bronchitis (Senior & Shapiro, *Pulmonary Diseases and Disorders*, 3d ed., New York, McGraw-Hill, 1998, pp. 659-681, 1998; Barnes, *Chest 117*, 10S-14S, 2000). Emphysema is characterized by destruction of alveolar walls leading to abnormal enlargement of the air spaces of the lung. Chronic bronchitis is defined clinically as the presence of chronic productive cough for three months in each of two successive years. In COPD, airflow obstruction is usually progressive and is only partially reversible. By far the most important risk factor for development of COPD is cigarette smoking, although the disease does occur in non-smokers.

Chronic inflammation of the airways is a key pathological feature of COPD (Senior & Shapiro, 1998). The inflammatory cell population comprises increased numbers of macrophages, neutrophils, and $CD8^+$ lymphocytes. Inhaled irritants, such as cigarette smoke, activate macrophages which are resident in the respiratory tract, as well as epithelial cells leading to release of chemokines (*e.g.*, interleukin-8) and other chemotactic factors. These chemotactic factors act to increase the neutrophil/monocyte trafficking from the blood into the lung tissue and airways. Neutrophils and monocytes recruited into the airways can release a variety of potentially damaging mediators such as proteolytic enzymes and reactive oxygen species. Matrix degradation and emphysema, along with airway wall thickening, surfactant dysfunction, and mucus hypersecretion, all are potential sequelae of this inflammatory response that lead to impaired airflow and gas exchange.

Protein kinases are signal transducing enzymes that phosphorylate proteins, including other kinases, and, along with protein phosphatases, regulate the level and extent of protein phosphorylation and activation. Intracellular signalling pathways have important roles in inflammatory processes. These pathways may be activated by cytokines, oxidant stress and other inflammatory mediators (reviewed in Kyraikis and Avruch, 1996 and 2001). For example, the pro-inflammatory cytokines, tumor

necrosis factor α (TNFα) and interleukin-1 activate the protein ser/thr kinases c-Jun-NH2-terminal kinase (JNK) and p38 mitogen-activated protein (MAP) kinase, leading to activation of AP-1 and IKB kinase (IKK), which, in turn, leads to activation of the transcription factor NFKB. Activation of NFKB is required for the transcription of several pro-inflammatory molecules, including interleukin-8 and ICAM-1. Enzymes of the MAP kinase class may also act to increase cytokine production by stabilization of mRNA (Winzen et al., 1999).

Inhibition of specific protein kinases has been shown to elicit anti-inflammatory effects. For example, the accumulation of polymorphonuclear leukocytes in murine lung following intratracheal administration of bacterial lipopolysaccharide can be blocked by inhibition of p38 MAP kinase (Nick, et al. 2000). As a further example, aerosol delivery to rat lungs of antisense oligodeoxynucleotides to syk kinase mRNA, suppressed nitric oxide and TNFα production from alveolar macrophages stimulated with IgG-anti-IgG complexes (Stenton et al. 2000). Protein kinase sub-types are therefore attractive therapeutic targets for the attenuation of the inflammatory response in COPD. See Kyriakis, J.M. and Avruch J. Sounding the alarm: protein kinase cascades activated by stress and inflammation. J Biol Chem 1996, **271**:24313-6; Kyriakis, J.M. and Avruch, J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. J. Physiol. Rev. 2001, **81**:807-69; Winzen, R., Kracht, M., Ritter, B., Wilhelm, A., Chen C.A., Shyu, A., Müller, M., Gaestel, M., Resch, K., and Holtmann, H. The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. EMBO J. 1999, **18**: 4969-4980; Nick, J.A., Young, S.K., Brown, K.K., Avdi, N.J., Arndt, P.G., Suratt, B.T., Janes, M.S., Henson, P.M., Worthen, G.S. Role of p38 mitogen-activated protein kinase in a murine model of pulmonary inflammation. J Immunol. 2000, **164**:2151-9; and Stenton, G.R., Kim, M.K., Nohara, O., Chen, C.F., Hirji, N., Wills, F.L., Gilchrist, M., Hwang, P.H., Park, J.G., Finlay, W., Jones, R.L., Befus, A.D., Schreiber, A.D. Aerosolized Syk antisense suppresses Syk expression,

mediator release from macrophages, and pulmonary inflammation. J Immunol 2000, **164**:3790-7.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or a CRIK polypeptide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects CRIK activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce CRIK activity. The reagent preferably binds to an expression product of a human CRIK gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells that have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a particular cell type, such as a cell-specific ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods that are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE

TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988); Wu *et al.*, *J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem. 266*, 338-42 (1991).

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases CRIK activity relative to the CRIK activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors that can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 μg to about 50 μg/kg, about 50 μg to about 5 mg/kg, about 100 μg to about 500 μg/kg of patient body weight, and about 200 to about 250 μg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg,

about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides that express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a CRIK gene or the activity of a CRIK polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a CRIK gene or the activity of a CRIK polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to CRIK-specific mRNA, quantitative RT-PCR, immunologic detection of a CRIK polypeptide, or measurement of CRIK activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate *therapeutic agents. Selection of the appropriate agents for use in combination* therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

Human CRIK also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences that encode the enzyme. For example, differences can be determined between the cDNA or genomic sequence encoding CRIK in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (*see, e.g.*, Myers *et al.*, *Science 230*, 1242, 1985). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g.*, Cotton *et al.*, *Proc. Natl. Acad. Sci. USA 85*, 4397-4401, 1985). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA

sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Altered levels of CRIK also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Detection of human citron rho/rac-interacting kinase activity

Subconfluent COS7 cells in 10-cm dishes are transiently transfected by the DEAE-dextran/chloroquine method with 10 μg of FLAG-SEQ ID NO: 1 vector. Cells are harvested 48 h after transfection. Immunoblotting is performed, and cells are probed with anti-FLAG M2 antibodies (Eastman Kodak Co.) Blots are developed using horseradish peroxidase-conjugated secondary antibodies and ECL detection system (Amersham Pharmacia Biotech). In vitro kinase assays are performed by incubating immune complexes in 50 ml of kinase buffer (50 mM HEPES, pH 7.4, 5 mM MgCl2, 3 mM MnCl2, 1mM dithiothreitol), in the presence or absence of 5 mg of histone H1 or myelin basic protein, plus 0.1 mM ATP and 10 mCi of [gamma-32P] ATP (6000 Ci/mM, NEN Life Science Products) for 30 min at 30 °C. The products are analyzed by 5% or 12.5% SDS-PAGE followed by autoradiography. For immunoprecipitation of metabolically labeled proteins, primary keratinocytes are

incubated with 0.1 mCi/ml [35S]methionine (Expre35S; NEN Life Science Products) for 4 h in methionine-free medium in the presence of serum. Immunoprecipitated proteins are separated on a 5% SDS-PAGE gel and visualized by autoradiography. It is shown that the polypeptide of SEQ ID NO: 2 has a human citron rho/rac-interacting kinase-short kinase activity.

EXAMPLE 2

Expression of recombinant human CRIK

The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to produce large quantities of recombinant human CRIK polypeptides in yeast. The CRIK-encoding DNA sequence is derived from SEQ ID NO:1. Before insertion into vector pPICZB, the DNA sequence is modified by well known methods in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an enterokinase cleavage site, a His6 reporter tag and a termination codon. Moreover, at both termini recognition sequences for restriction endonucleases are added and after digestion of the multiple cloning site of pPICZ B with the corresponding restriction enzymes the modified DNA sequence is ligated into pPICZB. This expression vector is designed for inducible expression in *Pichia pastoris*, driven by a yeast promoter. The resulting pPICZ/md-His6 vector is used to transform the yeast.

The yeast is cultivated under usual conditions in 5 liter shake flasks and the recombinantly produced protein isolated from the culture by affinity chromatography (Ni-NTA-Resin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human CRIK polypeptide is obtained.

EXAMPLE 3

Identification of test compounds that bind to CRIK polypeptides

Purified CRIK polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Human CRIK polypeptides comprise the amino acid sequence shown in SEQ ID NO:2. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a CRIK polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound that increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound is not incubated is identified as a compound which binds to a CRIK polypeptide.

EXAMPLE 4

Identification of a test compound which decreases CRIK gene expression

A test compound is administered to a culture of human cells transfected with a CRIK expression construct and incubated at 37 °C for 10 to 45 minutes. A culture of the same type of cells that have not been transfected is incubated for the same time without the test compound to provide a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 μg total RNA and hybridized with a ^{32}P-labeled CRIK-specific probe at 65 ° C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NO:1. A test compound that decreases the CRIK-

specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of CRIK gene expression.

EXAMPLE 5

Identification of a test compound which decreases CRIK activity

A test compound is administered to a culture of human cells transfected with a CRIK expression construct and incubated at 37 °C for 10 to 45 minutes. A culture of the same type of cells that have not been transfected is incubated for the same time without the test compound to provide a negative control. CRIK activity is measured using the method of Di Cunto *et al.*, J Biol Chem. 1998 Nov 6;273(45):29706-11.

A test compound which decreases the CRIK activity of the CRIK relative to the CRIK activity in the absence of the test compound is identified as an inhibitor of CRIK activity.

EXAMPLE 6

Tissue-specific expression of CRIK

The qualitative expression pattern of CRIK in various tissues is determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

To demonstrate that CRIK is involved in the disease process of COPD, the initial expression panel consists of RNA samples from respiratory tissues and inflammatory cells relevant to COPD: lung (adult and fetal), trachea, freshly isolated alveolar type II cells, cultured human bronchial epithelial cells, cultured small airway epithelial cells, cultured bronchial sooth muscle cells, cultured H441 cells (Clara-like), freshly isolated neutrophils and monocytes, and cultured monocytes (macrophage-like). Body map profiling also is carried out, using total RNA panels purchased from Clontech. The tissues are adrenal gland, bone marrow, brain, colon, heart, kidney,

liver, lung, mammary gland, pancreas, prostate, salivary gland, skeletal muscle, small intestine, spleen, stomach, testis, thymus, trachea, thyroid, and uterus.

To demonstrate that CRIK is involved in CNS disorders, the following tissues are screened: fetal and adult brain, muscle, heart, lung, kidney, liver, thymus, testis, colon, placenta, trachea, pancreas, kidney, gastric mucosa, colon, liver, cerebellum, skin, cortex (Alzheimer's and normal), hypothalamus, cortex, amygdala, cerebellum, hippocampus, choroid, plexus, thalamus, and spinal cord.

To demonstrate that CRIK is involved in the disease process of obesity, expression is determined in the following tissues: subcutaneous adipose tissue, mesenteric adipose tissue, adrenal gland, bone marrow, brain (cerebellum, spinal cord, cerebral cortex, caudate, medulla, substantia nigra, and putamen), colon, fetal brain, heart, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle small intestine, spleen, stomach, testes, thymus, thyroid trachea, and uterus. Neuroblastoma cell lines SK-Nr-Be (2), Hr, Sk-N-As, HTB-10, IMR-32, SNSY-5Y, T3, SK-N-D2, D283, DAOY, CHP-2, U87MG, BE(2)C, T986, KANTS, MO59K, CHP234, C6 (rat), SK-N-F1, SK-PU-DW, PFSK-1, BE(2)M17, and MCIXC also are tested for CRIK expression. As a final step, the expression of CRIK in cells derived from normal individuals with the expression of cells derived from obese individuals is compared.

Quantitative expression profiling. Quantitative expression profiling is performed by the form of quantitative PCR analysis called "kinetic analysis" firstly described in Higuchi *et al.*, *BioTechnology 10*, 413-17, 1992, and Higuchi *et al.*, *BioTechnology 11*, 1026-30, 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies.

If the amplification is performed in the presence of an internally quenched fluorescent oligonucleotide (TaqMan probe) complementary to the target sequence,

the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase and a fluorescent dye released in the medium (Holland *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 7276-80, 1991). Because the fluorescence emission will increase in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid *et al.*, *Genome Res. 6*, 986-94, 1996, and Gibson *et al.*, *Genome Res. 6*, 995-1001, 1996).

The amplification of an endogenous control can be performed to standardize the amount of sample RNA added to a reaction. In this kind of experiment, the control of choice is the 18S ribosomal RNA. Because reporter dyes with differing emission spectra are available, the target and the endogenous control can be independently quantified in the same tube if probes labeled with different dyes are used.

All "real time PCR" measurements of fluorescence are made in the ABI Prism 7700.

RNA extraction and cDNA preparation. Total RNA from the tissues listed above are used for expression quantification. RNAs labeled "from autopsy" were extracted from autoptic tissues with the TRIzol reagent (Life Technologies, MD) according to the manufacturer's protocol.

Fifty µg of each RNA were treated with DNase I for 1 hour at 37°C in the following reaction mix: 0.2 U/µl RNase-free DNase I (Roche Diagnostics, Germany); 0.4 U/µl RNase inhibitor (PE Applied Biosystems, CA); 10 mM Tris-HCl pH 7.9; 10mM $MgCl_2$; 50 mM NaCl; and 1 mM DTT.

After incubation, RNA is extracted once with 1 volume of phenol:chloroform:isoamyl alcohol (24:24:1) and once with chloroform, and precipitated with 1/10 volume of 3 M NaAcetate, pH5.2, and 2 volumes of ethanol.

Fifty μg of each RNA from the autoptic tissues are DNase treated with the DNA-free kit purchased from Ambion (Ambion, TX). After resuspension and spectrophotometric quantification, each sample is reverse transcribed with the TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA) according to the manufacturer's protocol. The final concentration of RNA in the reaction mix is 200ng/μL. Reverse transcription is carried out with 2.5μM of random hexamer primers.

TaqMan quantitative analysis. Specific primers and probe are designed according to the recommendations of PE Applied Biosystems; the probe can be labeled at the 5' end FAM (6-carboxy-fluorescein) and at the 3' end with TAMRA (6-carboxy-tetramethyl-rhodamine). Quantification experiments are performed on 10 ng of reverse transcribed RNA from each sample. Each determination is done in triplicate.

Total cDNA content is normalized with the simultaneous quantification (multiplex PCR) of the 18S ribosomal RNA using the Pre-Developed TaqMan Assay Reagents (PDAR) Control Kit (PE Applied Biosystems, CA).

The assay reaction mix is as follows: 1X final TaqMan Universal PCR Master Mix (from 2X stock) (PE Applied Biosystems, CA); 1X PDAR control – 18S RNA (from 20X stock); 300 nM forward primer; 900 nM reverse primer; 200 nM probe; 10 ng cDNA; and water to 25 μl.

Each of the following steps are carried out once: pre PCR, 2 minutes at 50 °C, and 10 minutes at 95 °C. The following steps are carried out 40 times: denaturation, 15 seconds at 95 °C, annealing/extension, 1 minute at 60 °C.

The experiment is performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR are processed as described in the ABI Prism 7700 user's manual in order to achieve better background subtraction as well as signal linearity with the starting target quantity.

EXAMPLE 7

Identification of test compound efficacy in a COPD animal model

Guinea pigs are exposed on a single occasion to tobacco smoke for 50 minutes. Animals are sacrificed between 10 minutes and 24 hour following the end of the exposure and their lungs placed in RNAlater™. The lung tissue is homogenized, and total RNA was extracted using a Qiagens RNeasy™ Maxi kit. Molecular Probes RiboGreen™ RNA quantitation method is used to quantify the amount of RNA in each sample.

Total RNA is reverse transcribed, and the resultant cDNA is used in a real-time polymerase chain reaction (PCR). The cDNA is added to a solution containing the sense and anti-sense primers and the 6-carboxy-tetramethyl-rhodamine labelled probe of the CRIK gene. Cyclophilin is used as the housekeeping gene. The expression of the CRIK gene is measured using the TaqMan real-time PCR system that generates an amplification curve for each sample. From this curve a threshold cycle value is calculated: the fractional cycle number at which the amount of amplified target reaches a fixed threshold. A sample containing many copies of the CRIK gene will reach this threshold earlier than a sample containing fewer copies. The threshold is set at 0.2, and the threshold cycle C_T is calculated from the amplification curve. The C_T value for the CRIK gene is normalized using the C_T value for the housekeeping gene.

Expression of the CRIK gene is increased by at least 3-fold between 10 minutes and 3 hours post tobacco smoke exposure compared to air exposed control animals.

Test compounds are evaluated as follows. Animals are pre-treated with a test compound between 5 minutes and 1 hour prior to the tobacco smoke exposure and they are then sacrificed up to 3 hours after the tobacco smoke exposure has been completed. Control animals are pre-treated with the vehicle of the test compound via

the route of administration chosen for the test compound. A test compound that reduces the tobacco smoke induced upregulation of CRIK gene relative to the expression seen in vehicle treated tobacco smoke exposed animals is identified as an inhibitor of CRIK gene expression.

EXAMPLE 8

Expression of human citron rho/rac-interacting kinase

Total RNA used for Taqman quantitative analysis were either purchased (Clontech,CA) or extracted from tissues using TRIzol reagent (Life Technologies, MD) according to a modified vendor protocol which utilizes the Rneasy protocol (Qiagen, CA). One hundred µg of each RNA were treated with DNase I using RNase free- DNase (Qiagen, CA) for use with RNeasy or QiaAmp columns.

After elution and quantitation with Ribogreen (Molecular Probes Inc., OR), each sample was reverse transcribed using the GibcoBRL Superscript II First Strand Synthesis System for RT-PCR according to vendor protocol (Life Technologies, MD). The final concentration of RNA in the reaction mix was 50ng/µL. Reverse transcription was performed with 50 ng of random hexamers.

Specific primers and probe were designed according to PE Applied Biosystems' Primer Express program recommendations and are listed below:

forward primer: 5'-(TCCAATTTTGATGAACCAGAGAAG)-3'
reverse primer: 5'-(AACCCCACAAACGGCAGTT)-3'
probe: SYBR Green

Quantitation experiments were performed on 25 ng of reverse transcribed RNA from each sample. 18S ribosomal RNA was measured as a control using the Pre-Developed TaqMan Assay Reagents (PDAR)(PE Applied Biosystems, CA). The assay reaction mix was as follows:

	final
TaqMan SYBR Green PCR Master Mix (2x) (PE Applied Biosystems, CA)	1x
Forward primer	300nM
Reverse primer	300nM
cDNA	25ng
Water to 25uL	

PCR conditions:

Once: 2' minutes at 50° C
10 minutes at 95°C

40cycles: 15 sec.at 95°C
1 minute at 60°C

The experiment was performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR were processed as described in the ABI Prism 7700 user's manual. Fold change was calculated using the delta-delta CT method with normalization to the 18S values. Relative expression was calculated by normalizing to 18s (D Ct), then making the highest expressing tissue 100 and everything else relative to it. Copy number conversion was performed without normalization using the formula Cn=10(Ct-40.007)/-3.623.

The results are shown in FIG. 21.

Human citron rho/rac-interacting kinase expressed in adipose and skeletal muscle could be regulated to increase insulin sensitivity.

EXAMPLE 9

In vivo testing of compounds/target validation

1. Pain:

Acute Pain

Acute pain is measured on a hot plate mainly in rats. Two variants of hot plate testing are used: In the classical variant animals are put on a hot surface (52 to 56 °C) and the latency time is measured until the animals show nocifensive behavior, such as stepping or foot licking. The other variant is an increasing temperature hot plate where the experimental animals are put on a surface of neutral temperature. Subsequently this surface is slowly but constantly heated until the animals begin to lick a hind paw. The temperature which is reached when hind paw licking begins is a measure for pain threshold.

Compounds are tested against a vehicle treated control group. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Persistent Pain

Persistent pain is measured with the formalin or capsaicin test, mainly in rats. A solution of 1 to 5% formalin or 10 to 100 µg capsaicin is injected into one hind paw of the experimental animal. After formalin or capsaicin application the animals show nocifensive reactions like flinching, licking and biting of the affected paw. The number of nocifensive reactions within a time frame of up to 90 minutes is a measure for intensity of pain.

Compounds are tested against a vehicle treated control group. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to formalin or capsaicin administration.

Neuropathic Pain

Neuropathic pain is induced by different variants of unilateral sciatic nerve injury mainly in rats. The operation is performed under anesthesia. The first variant of sciatic nerve injury is produced by placing loosely constrictive ligatures around the common sciatic nerve. The second variant is the tight ligation of about the half of the diameter of the common sciatic nerve. In the next variant, a group of models is used in which tight ligations or transections are made of either the L5 and L6 spinal nerves, or the L% spinal nerve only. The fourth variant involves an axotomy of two of the three terminal branches of the sciatic nerve (tibial and common peroneal nerves) leaving the remaining sural nerve intact whereas the last variant comprises the axotomy of only the tibial branch leaving the sural and common nerves uninjured. Control animals are treated with a sham operation.

Postoperatively, the nerve injured animals develop a chronic mechanical allodynia, cold allodynioa, as well as a thermal hyperalgesia. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA; Electronic von Frey System, Somedic Sales AB, Hörby, Sweden). Thermal hyperalgesia is measured by means of a radiant heat source (Plantar Test, Ugo Basile, Comerio, Italy), or by means of a cold plate of 5 to 10 °C where the nocifensive reactions of the affected hind paw are counted as a measure of pain intensity. A further test for cold induced pain is the counting of nocifensive reactions, or duration of nocifensive responses after plantar administration of acetone to the affected hind limb. Chronic pain in general is assessed by registering the circadanian rhythms in activity (Surjo and Arndt, Universität zu Köln, Cologne, Germany), and by scoring differences in gait (foot print patterns; FOOTPRINTS program, Klapdor et al., 1997. A low cost method to analyze footprint patterns. J. Neurosci. Methods 75, 49-54).

Compounds are tested against sham operated and vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Inflammatory Pain

Inflammatory pain is induced mainly in rats by injection of 0.75 mg carrageenan or complete Freund's adjuvant into one hind paw. The animals develop an edema with mechanical allodynia as well as thermal hyperalgesia. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA). Thermal hyperalgesia is measured by means of a radiant heat source (Plantar Test, Ugo Basile, Comerio, Italy, Paw thermal stimulator, G. Ozaki, University of California, USA). For edema measurement two methods are being used. In the first method, the animals are sacrificed and the affected hindpaws sectioned and weighed. The second method comprises differences in paw volume by measuring water displacement in a plethysmometer (Ugo Basile, Comerio, Italy).

Compounds are tested against uninflamed as well as vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Diabetic Neuropathic Pain

Rats treated with a single intraperitoneal injection of 50 to 80 mg/kg streptozotocin develop a profound hyperglycemia and mechanical allodynia within 1 to 3 weeks. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA).

Compounds are tested against diabetic and non-diabetic vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

2. Parkinson's disease

6-Hydroxydopamine (6-OH-DA) Lesion

Degeneration of the dopaminergic nigrostriatal and striatopallidal pathways is the central pathological event in Parkinson's disease. This disorder has been mimicked experimentally in rats using single/sequential unilateral stereotaxic injections of 6-OH-DA into the medium forebrain bundle (MFB).

Male Wistar rats (Harlan Winkelmann, Germany), weighing 200±250 g at the beginning of the experiment, are used. The rats are maintained in a temperature- and humidity-controlled environment under a 12 h light/dark cycle with free access to food and water when not in experimental sessions. The following in vivo protocols are approved by the governmental authorities. All efforts are made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques.

Animals are administered pargyline on the day of surgery (Sigma, St. Louis, MO, USA; 50 mg/kg i.p.) in order to inhibit metabolism of 6-OHDA by monoamine oxidase and desmethylimipramine HCl (Sigma; 25 mg/kg i.p.) in order to prevent uptake of 6-OHDA by noradrenergic terminals. Thirty minutes later the rats are anesthetized with sodium pentobarbital (50 mg/kg) and placed in a stereotaxic frame. In order to lesion the DA nigrostriatal pathway 4 µl of 0.01% ascorbic acid-saline containing 8 µg of 6-OHDA HBr (Sigma) are injected into the left medial fore-brain bundle at a rate of 1 µl/min (2.4 mm anterior, 1.49 mm lateral, -2.7 mm ventral to Bregma and the skull surface). The needle is left in place an additional 5 min to allow diffusion to occur.

Stepping Test

Forelimb akinesia is assessed three weeks following lesion placement using a modified stepping test protocol. In brief, the animals are held by the experimenter with one hand fixing the hindlimbs and slightly raising the hind part above the surface. One paw is touching the table, and is then moved slowly sideways (5 s for 1 m), first in the forehand and then in the backhand direction. The number of adjusting steps is counted for both paws in the backhand and forehand direction of movement. The sequence of testing is right paw forehand and backhand adjusting stepping, followed by left paw forehand and backhand directions. The test is repeated three times on three consecutive days, after an initial training period of three days prior to the first testing. Forehand adjusted stepping reveals no consistent differences between lesioned and healthy control animals. Analysis is therefore restricted to backhand adjusted stepping.

Balance Test

Balance adjustments following postural challenge are also measured during the stepping test sessions. The rats are held in the same position as described in the stepping test and, instead of being moved sideways, tilted by the experimenter towards the side of the paw touching the table. This maneuver results in loss of balance and the ability of the rats to regain balance by forelimb movements is scored on a scale ranging from 0 to 3. Score 0 is given for a normal forelimb placement. When the forelimb movement is delayed but recovery of postural balance detected, score 1 is given. Score 2 represents a clear, yet insufficient, forelimb reaction, as evidenced by muscle contraction, but lack of success in recovering balance, and score 3 is given for no reaction of movement. The test is repeated three times a day on each side for three consecutive days after an initial training period of three days prior to the first testing.

Staircase Test (Paw Reaching)

A modified version of the staircase test is used for evaluation of paw reaching behavior three weeks following primary and secondary lesion placement. Plexiglass test boxes with a central platform and a removable staircase on each side are used. The apparatus is designed such that only the paw on the same side at each staircase can be used, thus providing a measure of independent forelimb use. For each test the animals are left in the test boxes for 15 min. The double staircase is filled with 7 x 3 chow pellets (Precision food pellets, formula: P, purified rodent diet, size 45 mg; Sandown Scientific) on each side. After each test the number of pellets eaten (successfully retrieved pellets) and the number of pellets taken (touched but dropped) for each paw and the success rate (pellets eaten/pellets taken) are counted separately. After three days of food deprivation (12 g per animal per day) the animals are tested for 11 days. Full analysis is conducted only for the last five days.

MPTP treatment

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) causes degeneration of mesencephalic dopaminergic (DAergic) neurons in rodents, non-human primates, and humans and, in so doing, reproduces many of the symptoms of Parkinson's disease. MPTP leads to a marked decrease in the levels of dopamine and its metabolites, and in the number of dopaminergic terminals in the striatum as well as severe loss of the tyrosine hydroxylase (TH)-immunoreactive cell bodies in the substantia nigra, pars compacta.

In order to obtain severe and long-lasting lesions, and to reduce mortality, animals receive single injections of MPTP, and are then tested for severity of lesion 7–10 days later. Successive MPTP injections are administered on days 1, 2 and 3. Animals receive application of 4 mg/kg MPTP hydrochloride (Sigma) in saline once daily. All injections are intraperitoneal (i.p.) and the MPTP stock solution is frozen between injections. Animals are decapitated on day 11.

Immunohistology

At the completion of behavioral experiments, all animals are anaesthetized with 3 ml thiopental (1 g/40 ml i.p., Tyrol Pharma). The mice are perfused transcardially with 0.01 M PBS (pH 7.4) for 2 min, followed by 4% paraformaldehyde (Merck) in PBS for 15 min. The brains are removed and placed in 4% paraformaldehyde for 24 h at 4 °C. For dehydration they are then transferred to a 20% sucrose (Merck) solution in 0.1 M PBS at 4 °C until they sink. The brains are frozen in methylbutan at -20 °C for 2 min and stored at -70 °C. Using a sledge microtome (mod. 3800-Frigocut, Leica), 25 µm sections are taken from the genu of the corpus callosum (AP 1.7 mm) to the hippocampus (AP 21.8 mm) and from AP 24.16 to AP 26.72. Forty-six sections are cut and stored in assorters in 0.25 M Tris buffer (pH 7.4) for immunohistochemistry.

A series of sections is processed for free-floating tyrosine hydroxylase (TH) immunohistochemistry. Following three rinses in 0.1 M PBS, endogenous peroxidase activity is quenched for 10 min in 0.3% H_2O_2 ±PBS. After rinsing in PBS, sections are preincubated in 10% normal bovine serum (Sigma) for 5 min as blocking agent and transferred to either primary anti-rat TH rabbit antiserum (dilution 1:2000).

Following overnight incubation at room temperature, sections for TH immunoreactivity are rinsed in PBS (2 x10 min) and incubated in biotinylated anti-rabbit immunoglobulin G raised in goat (dilution 1:200) (Vector) for 90 min, rinsed repeatedly and transferred to Vectastain ABC (Vector) solution for 1 h. 3,.3' -Diaminobenzidine tetrahydrochloride (DAB; Sigma) in 0.1 M PBS, supplemented with 0.005% H_2O_2 , serves as chromogen in the subsequent visualization reaction. Sections are mounted on to gelatin-coated slides, left to dry overnight, counter-stained with hematoxylin dehydrated in ascending alcohol concentrations and cleared in butylacetate. Coverslips are mounted on entellan.

Rotarod Test

We use a modification of the procedure described by Rozas and Labandeira-Garcia (1997), with a CR-1 Rotamex system (Columbus Instruments, Columbus, OH) comprising an IBM-compatible personal computer, a CIO-24 data acquisition card, a control unit, and a four-lane rotarod unit. The rotarod unit consists of a rotating spindle (diameter 7.3 cm) and individual compartments for each mouse. The system software allows preprogramming of session protocols with varying rotational speeds (0–80 rpm). Infrared beams are used to detect when a mouse has fallen onto the base grid beneath the rotarod. The system logs the fall as the end of the experiment for that mouse, and the total time on the rotarod, as well as the time of the fall and all the set-up parameters, are recorded. The system also allows a weak current to be passed through the base grid, to aid training.

3. Dementia

The object recognition task

The object recognition task has been designed to assess the effects of experimental manipulations on the cognitive performance of rodents. A rat is placed in an open field, in which two identical objects are present. The rats inspects both objects during the first trial of the object recognition task. In a second trial, after a retention interval of for example 24 hours, one of the two objects used in the first trial, the 'familiar' object, and a novel object are placed in the open field. The inspection time at each of the objects is registered. The basic measures in the OR task is the time spent by a rat exploring the two object the second trial. Good retention is reflected by higher exploration times towards the novel than the 'familiar' object.

Administration of the putative cognition enhancer prior to the first trial predominantly allows assessment of the effects on acquisition, and eventually on consolidation processes. Administration of the testing compound after the first trial

allows to assess the effects on consolidation processes, whereas administration before the second trial allows to measure effects on retrieval processes.

The passive avoidance task

The passive avoidance task assesses memory performance in rats and mice. The inhibitory avoidance apparatus consists of a two-compartment box with a light compartment and a dark compartment. The two compartments are separated by a guillotine door that can be operated by the experimenter. A threshold of 2 cm separates the two compartments when the guillotine door is raised. When the door is open, the illumination in the dark compartment is about 2 lux. The light intensity is about 500 lux at the center of the floor of the light compartment.

Two habituation sessions, one shock session, and a retention session are given, separated by inter-session intervals of 24 hours. In the habituation sessions and the retention session the rat is allowed to explore the apparatus for 300 sec. The rat is placed in the light compartment, facing the wall opposite to the guillotine door. After an accommodation period of 15 sec. the guillotine door is opened so that all parts of the apparatus can be visited freely. Rats normally avoid brightly lit areas and will enter the dark compartment within a few seconds.

In the shock session the guillotine door between the compartments is lowered as soon as the rat has entered the dark compartment with its four paws, and a scrambled 1 mA footshock is administered for 2 sec. The rat is removed from the apparatus and put back into its home cage. The procedure during the retention session is identical to that of the habituation sessions.

The step-through latency, that is the first latency of entering the dark compartment (in sec.) during the retention session is an index of the memory performance of the animal; the longer the latency to enter the dark compartment, the better the retention is. A testing compound in given half an hour before the shock session, together with

1 mg*kg^{-1} scopolamine. Scopolamine impairs the memory performance during the retention session 24 hours later. If the test compound increases the enter latency compared with the scopolamine-treated controls, is likely to possess cognition enhancing potential.

The Morris water escape task

The Morris water escape task measures spatial orientation learning in rodents. It is a test system that has extensively been used to investigate the effects of putative therapeutic on the cognitive functions of rats and mice. The performance of an animal is assessed in a circular water tank with an escape platform that is submerged about 1 cm below the surface of the water. The escape platform is not visible for an animal swimming in the water tank. Abundant extra-maze cues are provided by the furniture in the room, including desks, computer equipment, a second water tank, the presence of the experimenter, and by a radio on a shelf that is playing softly.

The animals receive four trials during five daily acquisition sessions. A trial is started by placing an animal into the pool, facing the wall of the tank. Each of four starting positions in the quadrants north, east, south, and west is used once in a series of four trials; their order is randomized. The escape platform is always in the same position. A trial is terminated as soon as the animal had climbs onto the escape platform or when 90 seconds have elapsed, whichever event occurs first. The animal is allowed to stay on the platform for 30 seconds. Then it is taken from the platform and the next trial is started. If an animal did not find the platform within 90 seconds it is put on the platform by the experimenter and is allowed to stay there for 30 seconds. After the fourth trial of the fifth daily session, an additional trial is given as a probe trial: the platform is removed, and the time the animal spends in the four quadrants is measured for 30 or 60 seconds. In the probe trial, all animals start from the same start position, opposite to the quadrant where the escape platform had been positioned during acquisition.

Four different measures are taken to evaluate the performance of an animal during acquisition training: escape latency, traveled distance, distance to platform, and swimming speed. The following measures are evaluated for the probe trial: time (s) in quadrants and traveled distance (cm) in the four quadrants. The probe trial provides additional information about how well an animal learned the position of the escape platform. If an animal spends more time and swims a longer distance in the quadrant where the platform had been positioned during the acquisition sessions than in any other quadrant, one concludes that the platform position has been learned well.

In order to assess the effects of putative cognition enhancing compounds, rats or mice with specific brain lesions which impair cognitive functions, or animals treated with compounds such as scopolamine or MK-801, which interfere with normal learning, or aged animals which suffer from cognitive deficits, are used.

The T-maze spontaneous alternation task

The T-maze spontaneous alternation task (TeMCAT) assesses the spatial memory performance in mice. The start arm and the two goal arms of the T-maze are provided with guillotine doors which can be operated manually by the experimenter. A mouse is put into the start arm at the beginning of training. The guillotine door is closed. In the first trial, the 'forced trial', either the left or right goal arm is blocked by lowering the guillotine door. After the mouse has been released from the start arm, it will negotiate the maze, eventually enter the open goal arm, and return to the start position, where it will be confined for 5 seconds, by lowering the guillotine door. Then, the animal can choose freely between the left and right goal arm (all guillotine-doors opened) during 14 'free choice' trials. As soon a the mouse has entered one goal arm, the other one is closed. The mouse eventually returns to the start arm and is free to visit whichever go alarm it wants after having been confined to the start arm for 5 seconds. After completion of 14 free choice trials in one session, the animal is removed from the maze. During training, the animal is never handled.

The percent alternations out of 14 trials is calculated. This percentage and the total time needed to complete the first forced trial and the subsequent 14 free choice trials (in s) is analyzed. Cognitive deficits are usually induced by an injection of scopolamine, 30 min before the start of the training session. Scopolamine reduced the per-cent alternations to chance level, or below. A cognition enhancer, which is always administered before the training session, will at least partially, antagonize the scopolamine-induced reduction in the spontaneous alternation rate.

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CLAIMS

1. An isolated polynucleotide being selected from the group consisting of:
   a. a polynucleotide encoding a human citron rho/rac-interacting kinase polypeptide comprising an amino acid sequence selected form the group consisting of:
      i. amino acid sequences which are at least about 97% identical to the amino acid sequence shown in SEQ ID NO: 2; and
      ii. the amino acid sequence shown in SEQ ID NO: 2.
   b. a polynucleotide comprising the sequence of SEQ ID NOS: 1 or 24;
   c. a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b) and encodes a human citron rho/rac-interacting kinase polypeptide;
   d. a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes a human citron rho/rac-interacting kinase polypeptide; and
   e. a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes a human citron rho/rac-interacting kinase polypeptide.

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.

4. A substantially purified human citron rho/rac-interacting kinase polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a human citron rho/rac-interacting kinase polypeptide, wherein the method comprises the following steps:

a. culturing the host cell of claim 3 under conditions suitable for the expression of the human citron rho/rac-interacting kinase polypeptide; and

b. recovering the human citron rho/rac-interacting kinase polypeptide from the host cell culture.

6. A method for detection of a polynucleotide encoding a human citron rho/rac-interacting kinase polypeptide in a biological sample comprising the following steps:

a. hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b. detecting said hybridization complex.

7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8. A method for the detection of a polynucleotide of claim 1 or a human citron rho/rac-interacting kinase polypeptide of claim 4 comprising the steps of:

a. contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the human citron rho/rac-interacting kinase polypeptide and

b. detecting the interaction

9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a human citron rho/rac-interacting kinase, comprising the steps of:

a. contacting a test compound with any human citron rho/rac-interacting kinase polypeptide encoded by any polynucleotide of claim1;

b. detecting binding of the test compound to the human citron rho/rac-interacting kinase polypeptide, wherein a test compound which binds

to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a human citron rho/rac-interacting kinase.

11. A method of screening for agents which regulate the activity of a human citron rho/rac-interacting kinase, comprising the steps of:

a. contacting a test compound with a human citron rho/rac-interacting kinase polypeptide encoded by any polynucleotide of claim 1; and

b. detecting a human citron rho/rac-interacting kinase activity of the polypeptide, wherein a test compound which increases the human citron rho/rac-interacting kinase activity is identified as a potential therapeutic agent for increasing the activity of the human citron rho/rac-interacting kinase, and wherein a test compound which decreases the human citron rho/rac-interacting kinase activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the human citron rho/rac-interacting kinase.

12. A method of screening for agents which decrease the activity of a human citron rho/rac-interacting kinase, comprising the steps of:

a. contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of human citron rho/rac-interacting kinase.

13. A method of reducing the activity of human citron rho/rac-interacting kinase, comprising the steps of:

a. contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any human citron rho/rac-interacting kinase polypeptide of claim 4, whereby the activity of human citron rho/rac-interacting kinase is reduced.

14. A reagent that modulates the activity of a human citron rho/rac-interacting kinase polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.

15. A pharmaceutical composition, comprising:

a. the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

16. Use of the expression vector of claim 2 or the reagent of claim 14 in the preparation of a medicament for modulating the activity of a human citron rho/rac-interacting kinase in a disease.

17. Use of claim 16 wherein the disease is obesity, a CNS disorder or COPD.

Fig. 1

atgttgaagt tcaaatatgg agcgcggaat cctttggatg ctggtgctgc tgaacccatt 60
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Fig. 1 (continued)

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gacaagctca atgacctgga gaagaagcat gctatgcttg aaatgaatgc ccgaagctta 3540
cagcagaagc tggagactga acgagagctc aaacagaggc ttctggaaga gcaagccaaa 3600
ttacagcagc agatggacct gcagaaaaat cacattttcc gtctgactca aggactgcaa 3660
gaagctctag atcgggctga tctactgaag acagaaagaa gtgacttgga gtatcagctg 3720
gaaaacattc aggttctcta ttctcatgaa aaggtgaaaa tggaaggcac tatttctcaa 3780
caaaccaaac tcattgattt tctgcaagcc aaaatggacc aacctgctaa aaagaaaaag 3840
gttcctctgc agtacaatga gctgaagctg gccctggaga aggagaaagc tcgctgtgca 3900
gagctagagg aagcccttca gaagacccgc atcgagctcc ggtccgcccg ggaggaagct 3960
gcccaccgca aagcaacgga ccacccacac ccatccacgc cagccaccgc gaggcagcag 4020
atcgccatgt ccgccatcgt gcggtcgcca gagcaccagc ccagtgccat gagcctgctg 4080
gccccgccat ccagccgcag aaaggagtct tcaactccag aggaatttag tcggcgtctt 4140
aaggaacgca tgcaccacaa tattcctcac cgattcaacg taggactgaa catgcgagcc 4200
acaaagtgtg ctgtgtgtct ggataccgtg cactttggac gccaggcatc caaatgtctc 4260
gaatgtcagg tgatgtgtca ccccaagtgc tccacgtgct tgccagccac ctgcggcttg 4320
cctgctgaat atgccacaca cttcaccgag gccttctgcc gtgacaaaat gaactcccca 4380
ggtctccaga ccaaggagcc cagcagcagc ttgcacctgg aagggtggat gaaggtgccc 4440
aggaataaca aacgaggaca gcaaggctgg gacaggaagt acattgtcct ggagggatca 4500
aaagtcctca tttatgacaa tgaagccaga gaagctggac agaggccggt ggaagaattt 4560

Fig. 1 (continued)

gagctgtgcc ttcccgacgg ggatgtatct attcatggtg ccgttggtgc ttccgaactc 4620
gcaaatacag ccaaagcaga tgtcccatac atactgaaga tggaatctca cccgcacacc 4680
acctgctggc ccgggagaac cctctacttg ctagctccca gcttccctga caaacagcgc 4740
tgggtcaccg ccttagaatc agttgtcgca ggtgggagag tttctaggga aaaagcagaa 4800
gctgatgcta aactgcttgg aaactccctg ctgaaactgg aaggtgatga ccgtctagac 4860
atgaactgca cgctgccctt cagtgaccag gtggtgttgg tgggcaccga ggaagggctc 4920
tacgccctga atgtcttgaa aaactcccta acccatgtcc caggaattgg agcagtcttc 4980
caaatttata ttatcaagga cctggagaag ctactcatga tagcaggaga agagcgggca 5040
ctgtgtcttg tggacgtgaa gaaagtgaaa cagtccctgg cccagtccca cctgcctgcc 5100
cagcccgaca tctcacccaa catttttgaa gctgtcaagg gctgccactt gtttggggca 5160
ggcaagattg agaacgggct ctgcatctgt gcagccatgc ccagcaaagt cgtcattctc 5220
cgctacaacg aaaacctcag caaatactgc atccggaaag agatagagac ctcagagccc 5280
tgcagctgta tccacttcac caattacagt atcctcattg gaaccaataa attctacgaa 5340
atcgacatga agcagtacac gctcgaggaa ttcctggata agaatgacca ttccttggca 5400
cctgctgtgt ttgccgcctc ttccaacagc ttccctgtct caatcgtgca ggtgaacagc 5460
gcagggcagc gagaggagta cttgctgtgt ttccacgaat ttggagtgtt cgtggattct 5520
tacggaagac gtagccgcac agacgatctc aagtggagtc gcttaccttt ggcctttgcc 5580
tacagagaac cctatctgtt tgtgacccac ttcaactcac tcgaagtaat tgagatccag 5640
gcacgctcct cagcagggac ccctgcccga gcgtacctgg acatcccgaa cccgcgctac 5700
ctgggccctg ccatttcctc aggagcgatt tacttggcgt cctcatacca ggataaatta 5760
agggtcattt gctgcaaggg aaacctcgtg aaggagtccg gcactgaaca ccaccggggc 5820
ccgtccacct cccgcagcag ccccaacaag cgaggcccac ccacgtacaa cgagcacatc 5880
accaagcgcg tggcctccag cccagcgccg cccgaaggcc ccagccaccc gcgagagcca 5940
agcacacccc accgctaccg cgaggggcgg accgagctgc gcagggacaa gtctcctggc 6000
cgccccctgg agcgagagaa gtcccccggc cggatgctca gcacgcggag agagcggtcc 6060
cccgggaggc tgtttgaaga cagcagcagg ggccggctgc ctgcgggagc cgtgaggacc 6120
ccgctgtccc aggtgaacaa ggtctgggac cagtcttcag tataa 6165

Fig. 2

```
Met Leu Lys Phe Lys Tyr Gly Ala Arg Asn Pro Leu Asp Ala Gly Ala
 1               5                  10                  15
Ala Glu Pro Ile Ala Ser Arg Ala Ser Arg Leu Asn Leu Phe Phe Gln
            20                  25                  30
Gly Lys Pro Pro Phe Met Thr Gln Gln Gln Met Ser Pro Leu Ser Arg
        35                  40                  45
Glu Gly Ile Leu Asp Ala Leu Phe Val Leu Phe Glu Glu Cys Ser Gln
    50                  55                  60
Pro Ala Leu Met Lys Ile Lys His Val Ser Asn Phe Val Arg Lys Tyr
65                  70                  75                  80
Ser Asp Thr Ile Ala Glu Leu Gln Glu Leu Gln Pro Ser Ala Lys Asp
                85                  90                  95
Phe Glu Val Arg Ser Leu Val Gly Cys Gly His Phe Ala Glu Val Gln
            100                 105                 110
Val Val Arg Glu Lys Ala Thr Gly Asp Ile Tyr Ala Met Lys Val Met
        115                 120                 125
Lys Lys Lys Ala Leu Leu Ala Gln Glu Gln Val Ser Phe Phe Glu Glu
    130                 135                 140
Glu Arg Asn Ile Leu Ser Arg Ser Thr Ser Pro Trp Ile Pro Gln Leu
145                 150                 155                 160
Gln Tyr Ala Phe Gln Asp Lys Asn His Leu Tyr Leu Val Met Glu Tyr
                165                 170                 175
Gln Pro Gly Gly Asp Leu Leu Ser Leu Leu Asn Arg Tyr Glu Asp Gln
            180                 185                 190
Leu Asp Glu Asn Leu Ile Gln Phe Tyr Leu Ala Glu Leu Ile Leu Ala
        195                 200                 205
Val His Ser Val His Leu Met Gly Tyr Val His Arg Asp Ile Lys Pro
    210                 215                 220
Glu Asn Ile Leu Val Asp Arg Thr Gly His Ile Lys Leu Val Asp Phe
225                 230                 235                 240
Gly Ser Ala Ala Lys Met Asn Ser Asn Lys Met Val Asn Ala Lys Leu
                245                 250                 255
Pro Ile Gly Thr Pro Asp Tyr Met Ala Pro Glu Val Leu Thr Val Met
            260                 265                 270
Asn Gly Asp Gly Lys Gly Thr Tyr Gly Leu Asp Cys Asp Trp Trp Ser
        275                 280                 285
Val Gly Val Ile Ala Tyr Glu Met Ile Tyr Gly Arg Ser Pro Phe Ala
    290                 295                 300
```

Fig. 2 (continued)

```
Glu Gly Thr Ser Ala Arg Thr Phe Asn Asn Ile Met Asn Phe Gln Arg
305                 310                 315                 320
Phe Leu Lys Phe Pro Asp Asp Pro Lys Val Ser Ser Asp Phe Leu Asp
                325                 330                 335
Leu Ile Gln Ser Leu Leu Cys Gly Gln Lys Glu Arg Leu Lys Phe Glu
            340                 345                 350
Gly Leu Cys Cys His Pro Phe Phe Ser Lys Ile Asp Trp Asn Asn Ile
        355                 360                 365
Arg Asn Ser Pro Pro Pro Phe Val Pro Thr Leu Lys Ser Asp Asp Asp
    370                 375                 380
Thr Ser Asn Phe Asp Glu Pro Glu Lys Asn Ser Trp Val Ser Ser Ser
385                 390                 395                 400
Pro Cys Gln Leu Ser Pro Ser Gly Phe Ser Gly Glu Glu Leu Pro Phe
                405                 410                 415
Val Gly Phe Ser Tyr Ser Lys Ala Leu Gly Ile Leu Gly Arg Ser Glu
            420                 425                 430
Ser Val Val Ser Gly Leu Asp Ser Pro Ala Lys Thr Ser Ser Met Glu
        435                 440                 445
Lys Lys Leu Leu Ile Lys Ser Lys Glu Leu Gln Asp Ser Gln Asp Lys
    450                 455                 460
Cys His Lys Met Glu Gln Glu Met Thr Arg Leu His Arg Arg Val Ser
465                 470                 475                 480
Glu Val Glu Ala Val Leu Ser Gln Lys Glu Val Glu Leu Lys Ala Ser
                485                 490                 495
Glu Thr Gln Arg Ser Leu Leu Glu Gln Asp Leu Ala Thr Tyr Ile Thr
            500                 505                 510
Glu Cys Ser Ser Leu Lys Arg Ser Leu Glu Gln Ala Arg Met Glu Val
        515                 520                 525
Ser Gln Glu Asp Asp Lys Ala Leu Gln Leu Leu His Asp Ile Arg Glu
    530                 535                 540
Gln Ser Arg Lys Leu Gln Glu Ile Lys Glu Gln Glu Tyr Gln Ala Gln
545                 550                 555                 560
Val Glu Glu Met Arg Leu Met Met Asn Gln Leu Glu Glu Asp Leu Val
                565                 570                 575
Ser Ala Arg Arg Arg Ser Asp Leu Tyr Glu Ser Glu Leu Arg Glu Ser
            580                 585                 590
Arg Leu Ala Ala Glu Glu Phe Lys Arg Lys Ala Thr Glu Cys Gln His
        595                 600                 605
```

Fig. 2 (continued)

```
Lys Leu Leu Lys Ala Lys Asp Gln Gly Lys Pro Glu Val Gly Glu Tyr
    610                 615                 620
Ala Lys Leu Glu Lys Ile Asn Ala Glu Gln Gln Leu Lys Ile Gln Glu
625                 630                 635                 640
Leu Gln Glu Lys Leu Glu Lys Ala Val Lys Ala Ser Thr Glu Ala Thr
                645                 650                 655
Glu Leu Leu Gln Asn Ile Arg Gln Ala Lys Glu Arg Ala Glu Arg Glu
            660                 665                 670
Leu Glu Lys Leu Gln Asn Arg Glu Asp Ser Ser Glu Gly Ile Arg Lys
        675                 680                 685
Lys Leu Val Glu Ala Glu Glu Arg Arg His Ser Leu Glu Asn Lys Val
    690                 695                 700
Lys Arg Leu Glu Thr Met Glu Arg Arg Glu Asn Arg Leu Lys Asp Asp
705                 710                 715                 720
Ile Gln Thr Lys Ser Gln Gln Ile Gln Gln Met Ala Asp Lys Ile Leu
                725                 730                 735
Glu Leu Glu Glu Lys His Arg Glu Ala Gln Val Ser Ala Gln His Leu
            740                 745                 750
Glu Val His Leu Lys Gln Lys Glu Gln His Tyr Glu Glu Lys Ile Lys
        755                 760                 765
Val Leu Asp Asn Gln Ile Lys Lys Asp Leu Ala Asp Lys Glu Thr Leu
    770                 775                 780
Glu Asn Met Met Gln Arg His Glu Glu Glu Ala His Glu Lys Gly Lys
785                 790                 795                 800
Ile Leu Ser Glu Gln Lys Ala Met Ile Asn Ala Met Asp Ser Lys Ile
                805                 810                 815
Arg Ser Leu Glu Gln Arg Ile Val Glu Leu Ser Glu Ala Asn Lys Leu
            820                 825                 830
Ala Ala Asn Ser Ser Leu Phe Thr Gln Arg Asn Met Lys Ala Gln Glu
        835                 840                 845
Glu Met Ile Ser Glu Leu Arg Gln Gln Lys Phe Tyr Leu Glu Thr Gln
    850                 855                 860
Ala Gly Lys Leu Glu Ala Gln Asn Arg Lys Leu Glu Glu Gln Leu Glu
865                 870                 875                 880
Lys Ile Ser His Gln Asp His Ser Asp Lys Asn Arg Leu Leu Glu Leu
                885                 890                 895
```

Fig. 2 (continued)

```
Glu Thr Arg Leu Arg Glu Val Ser Leu Glu His Glu Glu Gln Lys Leu
            900                 905                 910
Glu Leu Lys Arg Gln Leu Thr Glu Leu Gln Leu Ser Leu Gln Glu Arg
        915                 920                 925
Glu Ser Gln Leu Thr Ala Leu Gln Ala Ala Arg Ala Ala Leu Glu Ser
    930                 935                 940
Gln Leu Arg Gln Ala Lys Thr Glu Leu Glu Glu Thr Thr Ala Glu Ala
945                 950                 955                 960
Glu Glu Glu Ile Gln Ala Leu Thr Ala His Arg Asp Glu Ile Gln Arg
                965                 970                 975
Lys Phe Asp Ala Leu Arg Asn Ser Cys Thr Val Ile Thr Asp Leu Glu
            980                 985                 990
Glu Gln Leu Asn Gln Leu Thr Glu Asp Asn Ala Glu Leu Asn Asn Gln
        995                 1000                1005
Asn Phe Tyr Leu Ser Lys Gln Leu Asp Glu Ala Ser Gly Ala Asn Asp
    1010                1015                1020
Glu Ile Val Gln Leu Arg Ser Glu Val Asp His Leu Arg Arg Glu Ile
1025                1030                1035                1040
Thr Glu Arg Glu Met Gln Leu Thr Ser Gln Lys Gln Thr Met Glu Ala
                1045                1050                1055
Leu Lys Thr Thr Cys Thr Met Leu Glu Glu Gln Val Met Asp Leu Glu
            1060                1065                1070
Ala Leu Asn Asp Glu Leu Leu Glu Lys Glu Arg Gln Trp Glu Ala Trp
        1075                1080                1085
Arg Ser Val Leu Gly Asp Glu Lys Ser Gln Phe Glu Cys Arg Val Arg
    1090                1095                1100
Glu Leu Gln Arg Met Leu Asp Thr Glu Lys Gln Ser Arg Ala Arg Ala
1105                1110                1115                1120
Asp Gln Arg Ile Thr Glu Ser Arg Gln Val Val Glu Leu Ala Val Lys
                1125                1130                1135
Glu His Lys Ala Glu Ile Leu Ala Leu Gln Gln Ala Leu Lys Glu Gln
            1140                1145                1150
Lys Leu Lys Ala Glu Ser Leu Ser Asp Lys Leu Asn Asp Leu Glu Lys
        1155                1160                1165
Lys His Ala Met Leu Glu Met Asn Ala Arg Ser Leu Gln Gln Lys Leu
    1170                1175                1180
Glu Thr Glu Arg Glu Leu Lys Gln Arg Leu Leu Glu Glu Gln Ala Lys
1185                1190                1195                1200
```

Fig. 2 (continued)

```
Leu Gln Gln Gln Met Asp Leu Gln Lys Asn His Ile Phe Arg Leu Thr
                1205                1210                1215
Gln Gly Leu Gln Glu Ala Leu Asp Arg Ala Asp Leu Leu Lys Thr Glu
            1220                1225                1230
Arg Ser Asp Leu Glu Tyr Gln Leu Glu Asn Ile Gln Val Leu Tyr Ser
        1235                1240                1245
His Glu Lys Val Lys Met Glu Gly Thr Ile Ser Gln Gln Thr Lys Leu
    1250                1255                1260
Ile Asp Phe Leu Gln Ala Lys Met Asp Gln Pro Ala Lys Lys Lys Lys
1265                1270                1275                1280
Val Pro Leu Gln Tyr Asn Glu Leu Lys Leu Ala Leu Glu Lys Glu Lys
                1285                1290                1295
Ala Arg Cys Ala Glu Leu Glu Glu Ala Leu Gln Lys Thr Arg Ile Glu
            1300                1305                1310
Leu Arg Ser Ala Arg Glu Glu Ala Ala His Arg Lys Ala Thr Asp His
        1315                1320                1325
Pro His Pro Ser Thr Pro Ala Thr Ala Arg Gln Gln Ile Ala Met Ser
    1330                1335                1340
Ala Ile Val Arg Ser Pro Glu His Gln Pro Ser Ala Met Ser Leu Leu
1345                1350                1355                1360
Ala Pro Pro Ser Ser Arg Arg Lys Glu Ser Ser Thr Pro Glu Glu Phe
                1365                1370                1375
Ser Arg Arg Leu Lys Glu Arg Met His His Asn Ile Pro His Arg Phe
            1380                1385                1390
Asn Val Gly Leu Asn Met Arg Ala Thr Lys Cys Ala Val Cys Leu Asp
        1395                1400                1405
Thr Val His Phe Gly Arg Gln Ala Ser Lys Cys Leu Glu Cys Gln Val
    1410                1415                1420
Met Cys His Pro Lys Cys Ser Thr Cys Leu Pro Ala Thr Cys Gly Leu
1425                1430                1435                1440
Pro Ala Glu Tyr Ala Thr His Phe Thr Glu Ala Phe Cys Arg Asp Lys
                1445                1450                1455
Met Asn Ser Pro Gly Leu Gln Thr Lys Glu Pro Ser Ser Ser Leu His
            1460                1465                1470
Leu Glu Gly Trp Met Lys Val Pro Arg Asn Asn Lys Arg Gly Gln Gln
        1475                1480                1485
```

Fig. 2 (continued)

```
Gly Trp Asp Arg Lys Tyr Ile Val Leu Glu Gly Ser Lys Val Leu Ile
    1490                1495                1500
Tyr Asp Asn Glu Ala Arg Glu Ala Gly Gln Arg Pro Val Glu Glu Phe
1505                1510                1515                1520
Glu Leu Cys Leu Pro Asp Gly Asp Val Ser Ile His Gly Ala Val Gly
                1525                1530                1535
Ala Ser Glu Leu Ala Asn Thr Ala Lys Ala Asp Val Pro Tyr Ile Leu
            1540                1545                1550
Lys Met Glu Ser His Pro His Thr Thr Cys Trp Pro Gly Arg Thr Leu
        1555                1560                1565
Tyr Leu Leu Ala Pro Ser Phe Pro Asp Lys Gln Arg Trp Val Thr Ala
    1570                1575                1580
Leu Glu Ser Val Val Ala Gly Gly Arg Val Ser Arg Glu Lys Ala Glu
1585                1590                1595                1600
Ala Asp Ala Lys Leu Leu Gly Asn Ser Leu Leu Lys Leu Glu Gly Asp
                1605                1610                1615
Asp Arg Leu Asp Met Asn Cys Thr Leu Pro Phe Ser Asp Gln Val Val
            1620                1625                1630
Leu Val Gly Thr Glu Glu Gly Leu Tyr Ala Leu Asn Val Leu Lys Asn
        1635                1640                1645
Ser Leu Thr His Val Pro Gly Ile Gly Ala Val Phe Gln Ile Tyr Ile
    1650                1655                1660
Ile Lys Asp Leu Glu Lys Leu Leu Met Ile Ala Gly Glu Glu Arg Ala
1665                1670                1675                1680
Leu Cys Leu Val Asp Val Lys Lys Val Lys Gln Ser Leu Ala Gln Ser
                1685                1690                1695
His Leu Pro Ala Gln Pro Asp Ile Ser Pro Asn Ile Phe Glu Ala Val
            1700                1705                1710
Lys Gly Cys His Leu Phe Gly Ala Gly Lys Ile Glu Asn Gly Leu Cys
        1715                1720                1725
Ile Cys Ala Ala Met Pro Ser Lys Val Val Ile Leu Arg Tyr Asn Glu
    1730                1735                1740
Asn Leu Ser Lys Tyr Cys Ile Arg Lys Glu Ile Glu Thr Ser Glu Pro
1745                1750                1755                1760
Cys Ser Cys Ile His Phe Thr Asn Tyr Ser Ile Leu Ile Gly Thr Asn
                1765                1770                1775
```

Fig. 2 (continued)

```
Lys Phe Tyr Glu Ile Asp Met Lys Gln Tyr Thr Leu Glu Glu Phe Leu
            1780                1785                1790
Asp Lys Asn Asp His Ser Leu Ala Pro Ala Val Phe Ala Ala Ser Ser
        1795                1800                1805
Asn Ser Phe Pro Val Ser Ile Val Gln Val Asn Ser Ala Gly Gln Arg
    1810                1815                1820
Glu Glu Tyr Leu Leu Cys Phe His Glu Phe Gly Val Phe Val Asp Ser
1825                1830                1835                1840
Tyr Gly Arg Arg Ser Arg Thr Asp Asp Leu Lys Trp Ser Arg Leu Pro
                1845                1850                1855
Leu Ala Phe Ala Tyr Arg Glu Pro Tyr Leu Phe Val Thr His Phe Asn
            1860                1865                1870
Ser Leu Glu Val Ile Glu Ile Gln Ala Arg Ser Ser Ala Gly Thr Pro
        1875                1880                1885
Ala Arg Ala Tyr Leu Asp Ile Pro Asn Pro Arg Tyr Leu Gly Pro Ala
    1890                1895                1900
Ile Ser Ser Gly Ala Ile Tyr Leu Ala Ser Ser Tyr Gln Asp Lys Leu
1905                1910                1915                1920
Arg Val Ile Cys Cys Lys Gly Asn Leu Val Lys Glu Ser Gly Thr Glu
                1925                1930                1935
His His Arg Gly Pro Ser Thr Ser Arg Ser Ser Pro Asn Lys Arg Gly
            1940                1945                1950
Pro Pro Thr Tyr Asn Glu His Ile Thr Lys Arg Val Ala Ser Ser Pro
        1955                1960                1965
Ala Pro Pro Glu Gly Pro Ser His Pro Arg Glu Pro Ser Thr Pro His
    1970                1975                1980
Arg Tyr Arg Glu Gly Arg Thr Glu Leu Arg Arg Asp Lys Ser Pro Gly
1985                1990                1995                2000
Arg Pro Leu Glu Arg Glu Lys Ser Pro Gly Arg Met Leu Ser Thr Arg
                2005                2010                2015
Arg Glu Arg Ser Pro Gly Arg Leu Phe Glu Asp Ser Ser Arg Gly Arg
            2020                2025                2030
Leu Pro Ala Gly Ala Val Arg Thr Pro Leu Ser Gln Val Asn Lys Val
        2035                2040                2045
Trp Asp Gln Ser Ser Val
    2050
```

Fig. 3

```
Met Leu Lys Phe Lys Tyr Gly Val Arg Asn Pro Pro Glu Ala Ser Ala
 1               5                  10                  15
Ser Glu Pro Ile Ala Ser Arg Ala Ser Arg Leu Asn Leu Phe Phe Gln
            20                  25                  30
Gly Lys Pro Pro Leu Met Thr Gln Gln Gln Met Ser Ala Leu Ser Arg
        35                  40                  45
Glu Gly Met Leu Asp Ala Leu Phe Ala Leu Phe Glu Glu Cys Ser Gln
    50                  55                  60
Pro Ala Leu Met Lys Met Lys His Val Ser Ser Phe Val Gln Lys Tyr
65                  70                  75                  80
Ser Asp Thr Ile Ala Glu Leu Arg Glu Leu Gln Pro Ser Ala Arg Asp
                85                  90                  95
Phe Glu Val Arg Ser Leu Val Gly Cys Gly His Phe Ala Glu Val Gln
            100                 105                 110
Val Val Arg Glu Lys Ala Thr Gly Asp Val Tyr Ala Met Lys Ile Met
        115                 120                 125
Lys Lys Lys Ala Leu Leu Ala Gln Glu Gln Val Ser Phe Phe Glu Glu
    130                 135                 140
Glu Arg Asn Ile Leu Ser Arg Ser Thr Ser Pro Trp Ile Pro Gln Leu
145                 150                 155                 160
Gln Tyr Ala Phe Gln Asp Lys Asn Asn Leu Tyr Leu Val Met Glu Tyr
                165                 170                 175
Gln Pro Gly Gly Asp Phe Leu Ser Leu Leu Asn Arg Tyr Glu Asp Gln
            180                 185                 190
Leu Asp Glu Ser Met Ile Gln Phe Tyr Leu Ala Glu Leu Ile Leu Ala
        195                 200                 205
Val His Ser Val His Gln Met Gly Tyr Val His Arg Asp Ile Lys Pro
    210                 215                 220
Glu Asn Ile Leu Ile Asp Arg Thr Gly Glu Ile Lys Leu Val Asp Phe
225                 230                 235                 240
Gly Ser Ala Ala Lys Met Asn Ser Asn Lys Val Asp Ala Lys Leu Pro
                245                 250                 255
Ile Gly Thr Pro Asp Tyr Met Ala Pro Glu Val Leu Thr Val Met Asn
            260                 265                 270
Glu Asp Arg Arg Gly Thr Tyr Gly Leu Asp Cys Asp Trp Trp Ser Val
        275                 280                 285
Gly Val Val Ala Tyr Glu Met Val Tyr Gly Lys Thr Pro Phe Thr Glu
    290                 295                 300
```

Fig. 3 (continued)

```
Gly Thr Ser Ala Arg Thr Phe Asn Asn Ile Met Asn Phe Gln Arg Phe
305                 310                 315                 320
Leu Lys Phe Pro Asp Asp Pro Lys Val Ser Ser Glu Leu Leu Asp Leu
                325                 330                 335
Leu Gln Ser Leu Leu Cys Val Gln Lys Glu Arg Leu Lys Phe Glu Gly
            340                 345                 350
Leu Cys Cys His Pro Phe Phe Ala Arg Thr Asp Trp Asn Asn Ile Arg
        355                 360                 365
Asn Ser Pro Pro Pro Phe Val Pro Thr Leu Lys Ser Asp Asp Asp Thr
    370                 375                 380
Ser Asn Phe Asp Glu Pro Glu Lys Asn Ser Trp Ala Phe Ile Leu Cys
385                 390                 395                 400
Val Pro Ala Glu Pro Leu Ala Phe Ser Gly Glu Glu Leu Pro Phe Val
                405                 410                 415
Gly Phe Ser Tyr Ser Lys Ala Leu Gly Tyr Leu Gly Arg Ser Glu Ser
            420                 425                 430
Val Val Ser Ser Leu Asp Ser Pro Ala Lys Val Ser Ser Met Glu Lys
        435                 440                 445
Lys Leu Leu Ile Lys Ser Lys Glu Leu Gln Asp Ser Gln Asp Lys Cys
    450                 455                 460
His Lys Met Glu Gln Glu Met Thr Arg Leu His Arg Arg Val Ser Glu
465                 470                 475                 480
Val Glu Ala Val Leu Ser Gln Lys Glu Val Glu Leu Lys Ala Ser Glu
                485                 490                 495
Thr Gln Arg Ser Leu Leu Glu Gln Asp Leu Ala Thr Tyr Ile Thr Glu
            500                 505                 510
Cys Ser Ser Leu Lys Arg Ser Leu Glu Gln Ala Arg Met Glu Val Ser
        515                 520                 525
Gln Glu Asp Asp Lys Ala Leu Gln Leu Leu His Asp Ile Arg Glu Gln
    530                 535                 540
Ser Arg Lys Leu Gln Glu Ile Lys Glu Gln Glu Tyr Gln Ala Gln Val
545                 550                 555                 560
Glu Glu Met Arg Leu Met Met Asn Gln Leu Glu Glu Asp Leu Val Ser
                565                 570                 575
Ala Arg Arg Arg Ser Asp Leu Tyr Glu Ser Glu Leu Arg Glu Ser Arg
            580                 585                 590
```

Fig. 3 (continued)

```
Leu Ala Ala Glu Glu Phe Lys Arg Lys Ala Asn Glu Cys Gln His Lys
        595                 600                 605
Leu Met Lys Ala Lys Asp Gln Gly Lys Pro Glu Val Gly Glu Tyr Ser
    610                 615                 620
Lys Leu Glu Lys Ile Asn Ala Glu Gln Gln Leu Lys Ile Gln Glu Leu
625                 630                 635                 640
Gln Glu Lys Leu Glu Lys Ala Val Lys Ala Ser Thr Glu Ala Thr Glu
                645                 650                 655
Leu Leu Gln Asn Ile Arg Gln Ala Lys Glu Arg Ala Glu Arg Glu Leu
            660                 665                 670
Glu Lys Leu His Asn Arg Glu Asp Ser Ser Glu Gly Ile Lys Lys Lys
        675                 680                 685
Leu Val Glu Ala Glu Glu Arg Arg His Ser Leu Glu Asn Lys Val Lys
    690                 695                 700
Arg Leu Glu Thr Met Glu Arg Arg Glu Asn Arg Leu Lys Asp Asp Ile
705                 710                 715                 720
Gln Thr Lys Ser Glu Gln Ile Gln Gln Met Ala Asp Lys Ile Leu Glu
                725                 730                 735
Leu Glu Glu Lys His Arg Glu Ala Gln Val Ser Ala Gln His Leu Glu
            740                 745                 750
Val His Leu Lys Gln Lys Glu Gln His Tyr Glu Glu Lys Ile Lys Val
        755                 760                 765
Leu Asp Asn Gln Ile Lys Lys Asp Leu Ala Asp Lys Glu Ser Leu Glu
    770                 775                 780
Asn Met Met Gln Arg His Glu Glu Glu Ala His Glu Lys Gly Lys Ile
785                 790                 795                 800
Leu Ser Glu Gln Lys Ala Met Ile Asn Ala Met Asp Ser Lys Ile Arg
                805                 810                 815
Ser Leu Glu Gln Arg Ile Val Glu Leu Ser Glu Ala Asn Lys Leu Ala
            820                 825                 830
Ala Asn Ser Ser Leu Phe Thr Gln Arg Asn Met Lys Ala Gln Glu Glu
        835                 840                 845
Met Ile Ser Glu Leu Arg Gln Gln Lys Phe Tyr Leu Glu Thr Gln Ala
    850                 855                 860
Gly Lys Leu Glu Ala Gln Asn Arg Lys Leu Glu Glu Gln Leu Glu Lys
865                 870                 875                 880
Ile Ser His Gln Asp His Ser Asp Lys Ser Arg Leu Leu Glu Leu Glu
                885                 890                 895
```

Fig. 3 (continued)

```
Thr Arg Leu Arg Glu Val Ser Leu Glu His Glu Glu Gln Lys Leu Glu
            900                 905                 910
Leu Lys Arg Gln Leu Thr Glu Leu Gln Leu Ser Leu Gln Glu Arg Glu
        915                 920                 925
Ser Gln Leu Thr Ala Leu Gln Ala Ala Arg Ala Ala Leu Glu Ser Gln
    930                 935                 940
Leu Arg Gln Ala Lys Thr Glu Leu Glu Glu Thr Thr Ala Glu Ala Glu
945                 950                 955                 960
Glu Glu Ile Gln Ala Leu Thr Ala His Arg Asp Glu Ile Gln Arg Lys
                965                 970                 975
Phe Asp Ala Leu Arg Asn Ser Cys Thr Val Ile Thr Asp Leu Glu Glu
            980                 985                 990
Gln Leu Asn Gln Leu Thr Glu Asp Asn Ala Glu Leu Asn Asn Gln Asn
        995                 1000                1005
Phe Tyr Leu Ser Lys Gln Leu Asp Glu Ala Ser Gly Ala Asn Asp Glu
    1010                1015                1020
Ile Val Gln Leu Arg Ser Glu Val Asp His Leu Arg Arg Glu Ile Thr
1025                1030                1035                1040
Glu Arg Glu Met Gln Leu Thr Ser Gln Lys Gln Thr Met Glu Ala Leu
                1045                1050                1055
Lys Thr Thr Cys Thr Met Leu Glu Glu Gln Val Leu Asp Leu Glu Ala
            1060                1065                1070
Leu Asn Asp Glu Leu Leu Glu Lys Glu Arg Gln Trp Glu Ala Trp Arg
        1075                1080                1085
Ser Val Leu Gly Asp Glu Lys Ser Gln Phe Glu Cys Arg Val Arg Glu
    1090                1095                1100
Leu Gln Arg Met Leu Asp Thr Glu Lys Gln Ser Arg Ala Arg Ala Asp
1105                1110                1115                1120
Gln Arg Ile Thr Glu Ser Arg Gln Val Val Glu Leu Ala Val Lys Glu
                1125                1130                1135
His Lys Ala Glu Ile Leu Ala Leu Gln Gln Ala Leu Lys Glu Gln Lys
            1140                1145                1150
Leu Lys Ala Glu Ser Leu Ser Asp Lys Leu Asn Asp Leu Glu Lys Lys
        1155                1160                1165
His Ala Met Leu Glu Met Asn Ala Arg Ser Leu Gln Gln Lys Leu Glu
    1170                1175                1180
```

Fig. 3 (continued)

```
Thr Glu Arg Glu Leu Lys Gln Arg Leu Leu Glu Glu Gln Ala Lys Leu
1185                1190                1195                1200
Gln Gln Gln Met Asp Leu Gln Lys Asn His Ile Phe Arg Leu Thr Gln
                1205                1210                1215
Gly Leu Gln Glu Ala Leu Asp Arg Ala Asp Leu Leu Lys Thr Glu Arg
            1220                1225                1230
Ser Asp Leu Glu Tyr Gln Leu Glu Asn Ile Gln Val Leu Tyr Ser His
        1235                1240                1245
Glu Lys Val Lys Met Glu Gly Thr Ile Ser Gln Gln Thr Lys Leu Ile
    1250                1255                1260
Asp Phe Leu Gln Ala Lys Met Asp Gln Pro Ala Lys Lys Lys Lys Val
1265                1270                1275                1280
Pro Leu Gln Tyr Asn Glu Leu Lys Leu Ala Leu Glu Lys Glu Lys Ala
                1285                1290                1295
Arg Cys Ala Glu Leu Glu Glu Ala Leu Gln Lys Thr Arg Ile Glu Leu
            1300                1305                1310
Arg Ser Ala Arg Glu Glu Ala Ala His Arg Lys Ala Thr Asp His Pro
        1315                1320                1325
His Pro Ser Thr Pro Ala Thr Ala Arg Gln Gln Ile Ala Met Ser Ala
    1330                1335                1340
Ile Val Arg Ser Pro Glu His Gln Pro Ser Ala Met Ser Leu Leu Ala
1345                1350                1355                1360
Pro Pro Ser Ser Arg Arg Lys Glu Ser Ser Thr Pro Glu Glu Phe Ser
                1365                1370                1375
Arg Arg Leu Lys Glu Arg Met His His Asn Ile Pro His Arg Phe Asn
            1380                1385                1390
Val Gly Leu Asn Met Arg Ala Thr Lys Cys Ala Val Cys Leu Asp Thr
        1395                1400                1405
Val His Phe Gly Arg Gln Ala Ser Lys Cys Leu Glu Cys Gln Val Met
    1410                1415                1420
Cys His Pro Lys Cys Ser Thr Cys Leu Pro Ala Thr Cys Gly Leu Pro
1425                1430                1435                1440
Ala Glu Tyr Ala Thr His Phe Thr Glu Ala Phe Cys Arg Asp Lys Met
                1445                1450                1455
Asn Ser Pro Gly Leu Gln Ser Lys Glu Pro Gly Ser Ser Leu His Leu
            1460                1465                1470
```

Fig. 3 (continued)

```
Glu Gly Trp Met Lys Val Pro Arg Asn Asn Lys Arg Gly Gln Gln Gly
        1475                1480                1485
Trp Asp Arg Lys Tyr Ile Val Leu Glu Gly Ser Lys Val Leu Ile Tyr
    1490                1495                1500
Asp Asn Glu Ala Arg Glu Ala Gly Gln Arg Pro Val Glu Glu Phe Glu
1505                1510                1515                1520
Leu Cys Leu Pro Asp Gly Asp Val Ser Ile His Gly Ala Val Gly Ala
                1525                1530                1535
Ser Glu Leu Ala Asn Thr Ala Lys Ala Asp Val Pro Tyr Ile Leu Lys
            1540                1545                1550
Met Glu Ser His Pro His Thr Thr Cys Trp Pro Gly Arg Thr Leu Tyr
        1555                1560                1565
Leu Leu Ala Pro Ser Phe Pro Asp Lys Gln Arg Trp Val Thr Ala Leu
    1570                1575                1580
Glu Ser Val Val Ala Gly Gly Arg Val Ser Arg Glu Lys Ala Glu Ala
1585                1590                1595                1600
Asp Ala Lys Leu Leu Gly Asn Ser Leu Leu Lys Leu Glu Gly Asp Asp
                1605                1610                1615
Arg Leu Asp Met Asn Cys Thr Leu Pro Phe Ser Asp Gln Val Val Leu
            1620                1625                1630
Val Gly Thr Glu Glu Gly Leu Tyr Ala Leu Asn Val Leu Lys Asn Ser
        1635                1640                1645
Leu Thr His Ile Pro Gly Ile Gly Ala Val Phe Gln Ile Tyr Ile Ile
    1650                1655                1660
Lys Asp Leu Glu Lys Leu Leu Met Ile Ala Gly Glu Glu Arg Ala Leu
1665                1670                1675                1680
Cys Leu Val Asp Val Lys Lys Val Lys Gln Ser Leu Ala Gln Ser His
                1685                1690                1695
Leu Pro Ala Gln Pro Asp Val Ser Pro Asn Ile Phe Glu Ala Val Lys
            1700                1705                1710
Gly Cys His Leu Phe Ala Ala Gly Lys Ile Glu Asn Ser Leu Cys Ile
        1715                1720                1725
Cys Ala Ala Met Pro Ser Lys Val Val Ile Leu Arg Tyr Asn Asp Asn
    1730                1735                1740
Leu Ser Lys Tyr Cys Ile Arg Lys Glu Ile Glu Thr Ser Glu Pro Cys
1745                1750                1755                1760
```

Fig. 3 (continued)

```
Ser Cys Ile His Phe Thr Asn Tyr Ser Ile Leu Ile Gly Thr Asn Lys
                1765                1770                1775
Phe Tyr Glu Ile Asp Met Lys Gln Tyr Thr Leu Asp Glu Phe Leu Asp
            1780                1785                1790
Lys Asn Asp His Ser Leu Ala Pro Ala Val Phe Ala Ser Ser Ser Asn
        1795                1800                1805
Ser Phe Pro Val Ser Ile Val Gln Ala Asn Ser Ala Gly Gln Arg Glu
    1810                1815                1820
Glu Tyr Leu Leu Cys Phe His Glu Phe Gly Val Phe Val Asp Ser Tyr
1825                1830                1835                1840
Gly Arg Arg Ser Arg Thr Asp Asp Leu Lys Trp Ser Arg Leu Pro Leu
                1845                1850                1855
Ala Phe Ala Tyr Arg Glu Pro Tyr Leu Phe Val Thr His Phe Asn Ser
            1860                1865                1870
Leu Glu Val Ile Glu Ile Gln Ala Arg Ser Ser Leu Gly Ser Pro Ala
        1875                1880                1885
Arg Ala Tyr Leu Glu Ile Pro Asn Pro Arg Tyr Leu Gly Pro Ala Ile
    1890                1895                1900
Ser Ser Gly Ala Ile Tyr Leu Ala Ser Ser Tyr Gln Asp Lys Leu Arg
1905                1910                1915                1920
Val Ile Cys Cys Lys Gly Asn Leu Val Lys Glu Ser Gly Thr Glu Gln
                1925                1930                1935
His Arg Val Pro Ser Thr Ser Arg Ser Ser Pro Asn Lys Arg Gly Pro
            1940                1945                1950
Pro Thr Tyr Asn Glu His Ile Thr Lys Arg Val Ala Ser Ser Pro Ala
        1955                1960                1965
Pro Pro Glu Gly Pro Ser His Pro Arg Glu Pro Ser Thr Pro His Arg
    1970                1975                1980
Tyr Arg Asp Arg Glu Gly Arg Thr Glu Leu Arg Arg Asp Lys Ser Pro
1985                1990                1995                2000
Gly Arg Pro Leu Glu Arg Glu Lys Ser Pro Gly Arg Met Leu Ser Thr
                2005                2010                2015
Arg Arg Glu Arg Ser Pro Gly Arg Leu Phe Glu Asp Ser Ser Arg Gly
            2020                2025                2030
Arg Leu Pro Ala Gly Ala Val Arg Thr Pro Leu Ser Gln Val Asn Lys
        2035                2040                2045
Val Trp Asp Gln Ser Ser Val
    2050                2055
```

Fig. 4

```
atgttgaagt tcaaatatgg agcgcggaat cctttggatg ctggtgctgc tgaacccatt      60
gccagccggg cctccaggct gaatctgttc ttccagggga aaccaccctt tatgactcaa     120
cagcagatgt ctcctctttc ccgagaaggg atattagatg ccctctttgt tctctttgaa     180
gaatgcagtc agcctgctct gatgaagatt aagcacgtga gcaactttgt ccggaagtat     240
tccgacacca tagctgagtt acaggagctc cagccttcgg caaaggactt cgaagtcaga     300
agtcttgtag gttgtggtca ctttgctgaa gtgcaggtgg taagagagaa agcaaccggg     360
gacatctatg ctatgaaagt gatgaagaag aaggctttat tggcccagga gcaggtttca     420
ttttttgagg aagagcggaa catattatct cgaagcacaa gcccgtggat cccccaatta     480
cagtatgcct ttcaggacaa aaatcacctt tatctggtca tggaatatca gcctggaggg     540
gacttgctgt cacttttgaa tagatatgag gaccagttag atgaaaacct gatacagttt     600
tacctagctg agctgatttt ggctgttcac agcgttcatc tgatgggata cgtgcatcga     660
gacatcaagc ctgagaacat tctcgttgac cgcacaggac acatcaagct ggtggatttt     720
ggatctgccg cgaaaatgaa ttcaaacaag atggtgaatg ccaaactccc gattgggacc     780
ccagattaca tggctcctga agtgctgact gtgatgaacg gggatggaaa aggcacctac     840
ggcctggact gtgactggtg gtcagtgggc gtgattgcct atgagatgat ttatgggaga     900
tccccttcg cagagggaac ctctgccaga accttcaata acattatgaa tttccagcgg      960
tttttgaaat ttccagatga ccccaaagtg agcagtgact ttcttgatct gattcaaagc    1020
ttgttgtgcg gccagaaaga gagactgaag tttgaaggtc tttgctgcca tcctttcttc    1080
tctaaaattg actggaacaa cattcgtaac tctcctcccc ccttcgttcc caccctcaag    1140
tctgacgatg acacctccaa ttttgatgaa ccagagaaga attcgtgggt ttcatcctct    1200
ccgtgccagc tgagcccctc aggcttctcg ggtgaagaac tgccgtttgt ggggttttcg    1260
tacagcaagg cactggggat tcttggtaga tctgagtctg ttgtgtcggg tctggactcc    1320
cctgccaaga ctagctccat ggaaaagaaa cttctcatca aaagcaaaga gctacaagac    1380
tctcaggaca agtgtcacaa gatggagcag gaaatgaccc ggttacatcg gagagtgtca    1440
gaggtggagg ctgtgcttag tcagaaggag gtggagctga aggcctctga gactcagaga    1500
tccctcctgg agcaggacct tgctacctac atcacagaat gcagtagctt aaagcgaagt    1560
ttggagcaag cacggatgga ggtgtcccag gaggatgaca aagcactgca gcttctccat    1620
gatatcagag agcagagccg gaagctccaa gaaatcaaag agcaggagta ccaggctcaa    1680
gtggaagaaa tgaggttgat gatgaatcag ttggaagagg atcttgtctc agcaagaaga    1740
cggagtgatc tctacgaatc tgagctgaga gagtctcggc ttgctgctga agaattcaag    1800
cggaaagcga cagaatgtca gcataaactg ttgaaggcta aggatcaagg gaagcctgaa    1860
gtgggagaat atgcgaaact ggagaagatc aatgctgagc agcagctcaa aattcaggag    1920
ctccaagaga aactggagaa ggctgtaaaa gccagcacgg aggccaccga gctgctgcag    1980
aatatccgcc aggcaaagga gcgagccgag agggagctgg agaagctgca gaaccgagag    2040
gattcttctg aaggcatcag aaagaagctg gtggaagctg aggaacgccg ccattctctg    2100
gagaacaagg taaagagact agagaccatg gagcgtagag aaaacagact gaaggatgac    2160
atccagacaa aatcccaaca gatccagcag atggctgata aaattctgga gctcgaagag    2220
aaacatcggg aggcccaagt ctcagcccag cacctagaag tgcacctgaa acagaaagag    2280
```

Fig. 4 (continued)

```
cagcactatg aggaaaagat taaagtgttg gacaatcaga taaagaaaga cctggctgac   2340
aaggagacac tggagaacat gatgcagaga cacgaggagg aggcccatga gaagggcaaa   2400
attctcagcg aacagaaggc gatgatcaat gctatggatt ccaagatcag atccctggaa   2460
cagaggattg tggaactgtc tgaagccaat aaacttgcag caaatagcag tctttttacc   2520
caaaggaaca tgaaggccca agaagagatg atttctgaac tcaggcaaca gaaattttac   2580
ctggagacac aggctgggaa gttggaggcc cagaaccgaa aactggagga gcagctggag   2640
aagatcagcc accaagacca cagtgacaag aatcggctgc tggaactgga gacaagattg   2700
cgggaggtca gtctagagca cgaggagcag aaactggagc tcaagcgcca gctcacagag   2760
ctacagctct ccctgcagga gcgcgagtca cagttgacag ccctgcaggc tgcacgggcg   2820
gccctggaga gccagcttcg ccaggcgaag acagagctgg aagagaccac agcagaagct   2880
gaagaggaga tccaggcact cacggcacat agagatgaaa tccagcgcaa atttgatgct   2940
cttcgtaaca gctgtactgt aatcacagac ctggaggagc agctaaacca gctgaccgag   3000
gacaacgctg aactcaacaa ccaaaacttc tacttgtcca aacaactcga tgaggcttct   3060
ggcgccaacg acgagattgt acaactgcga agtgaagtgg accatctccg ccgggagatc   3120
acggaacgag agatgcagct taccagccag aagcaaacga tggaggctct gaagaccacg   3180
tgcaccatgc tggaggaaca ggtcatggat ttggaggccc taaacgatga gctgctagaa   3240
aaagagcggc agtgggaggc ctggaggagc gtcctgggtg atgagaaatc ccagtttgag   3300
tgtcgggttc gagagctgca gagaatgctg gacaccgaga aacagagcag ggcgagagcc   3360
gatcagcgga tcaccgagtc tcgccaggtg gtggagctgg cagtgaagga gcacaaggct   3420
gagattctcg ctctgcagca ggctctcaaa gagcagaagc tgaaggccga gagcctctct   3480
gacaagctca atgacctgga gaagaagcat gctatgcttg aaatgaatgc ccgaagctta   3540
cagcagaagc tggagactga acgagagctc aaacagaggc ttctggaaga gcaagccaaa   3600
ttacagcagc agatggacct gcagaaaaat cacattttcc gtctgactca aggactgcaa   3660
gaagctctag atcgggctga tctactgaag acagaaagaa gtgacttgga gtatcagctg   3720
gaaaacattc aggttctcta ttctcatgaa aaggtgaaaa tggaaggcac tatttctcaa   3780
caaaccaaac tcattgattt tctgcaagcc aaaatggacc aacctgctaa aaagaaaaag   3840
gttcctctgc agtacaatga gctgaagctg gccctggaga aggagaaagc tcgctgtgca   3900
gagctagagg aagcccttca gaagacccgc atcgagctcc ggtccgcccg ggaggaagct   3960
gcccaccgca aagcaacgga ccacccacac ccatccacgc cagccaccgc gaggcagcag   4020
atcgccatgt ccgccatcgt gcggtcgcca gagcaccagc ccagtgccat gagcctgctg   4080
gccccgccat ccagccgcag aaaggagtct tcaactccag aggaatttag tcggcgtctt   4140
aaggaacgca tgcaccacaa tattcctcac cgattcaacg taggactgaa catgcgagcc   4200
acaaagtgtg ctgtgtgtct ggataccgtg cactttggac gccaggcatc caaatgtctc   4260
gaatgtcagg tgatgtgtca ccccaagtgc tccacgtgct tgccagccac ctgcggcttg   4320
cctgctgaat atgccacaca cttcaccgag gccttctgcc gtgacaaaat gaactcccca   4380
ggtctccaga ccaaggagcc cagcagcagc ttgcacctgg aagggtggat gaaggtgccc   4440
aggaataaca aacgaggaca gcaaggctgg gacaggaagt acattgtcct ggagggatca   4500
aaagtcctca tttatgacaa tgaagccaga gaagctggac agaggccggt ggaagaattt   4560
```

Fig. 4 (continued)

gagctgtgcc ttcccgacgg ggatgtatct attcatggtg ccgttggtgc ttccgaactc 4620
gcaaatacag ccaaagcaga tgtcccatac atactgaaga tggaatctca cccgcacacc 4680
acctgctggc ccgggagaac cctctacttg ctagctccca gcttccctga caaacagcgc 4740
tgggtcaccg ccttagaatc agttgtcgca ggtgggagag tttctaggga aaaagcagaa 4800
gctgatgcta aactgcttgg aaactccctg ctgaaactgg aaggtgatga ccgtctagac 4860
atgaactgca cgctgccctt cagtgaccag gtggtgttgg tgggcaccga ggaagggctc 4920
tacgccctga atgtcttgaa aaactcccta acccatgtcc caggaattgg agcagtcttc 4980
caaatttata ttatcaagga cctggagaag ctactcatga tagcaggaga agagcgggca 5040
ctgtgtcttg tggacgtgaa gaaagtgaaa cagtccctgg cccagtccca cctgcctgcc 5100
cagcccgaca tctcacccaa cattttttgaa gctgtcaagg gctgccactt gtttggggca 5160
ggcaagattg agaacgggct ctgcatctgt gcagccatgc ccagcaaagt cgtcattctc 5220
cgctacaacg aaaacctcag caaatactgc atccggaaag agatagagac ctcagagccc 5280
tgcagctgta tccacttcac caattacagt atcctcattg gaaccaataa attctacgaa 5340
atcgacatga agcagtacac gctcgaggaa ttcctggata agaatgacca ttccttggca 5400
cctgctgtgt ttgccgcctc ttccaacagc ttccctgtct caatcgtgca ggtgaacagc 5460
gcagggcagc gagaggagta cttgctgtgt ttccacgaat ttggagtgtt cgtggattct 5520
tacggaagac gtagccgcac agacgatctc aagtggagtc gcttaccttt ggcctttgcc 5580
tacagagaac cctatctgtt tgtgacccac ttcaactcac tcgaagtaat tgagatccag 5640
gcacgctcct cagcagggac ccctgcccga gcgtacctgg acatcccgaa cccgcgctac 5700
ctgggccctg ccatttcctc aggagcgatt tacttggcgt cctcatacca ggataaatta 5760
agggtcattt gctgcaaggg aaacctcgtg aaggagtccg gcactgaaca ccaccggggc 5820
ccgtccacct cccgcagcag ccccaacaag cgaggcccac ccacgtacaa cgagcacatc 5880
accaagcgcg tggcctccag cccagcgccg cccgaaggcc ccagccaccc gcgagagcca 5940
agcacacccc accgctaccg cgaggggcgg accgagctgc gcagggacaa gtctcctggc 6000
cgccccctgg agcgagagaa gtcccccggc cggatgctca gcacgcggag agagcggtcc 6060
cccgggaggc tgtttgaaga cagcagcagg ggccggctgc ctgcgggagc cgtgaggacc 6120
ccgctgtccc aggtgaacaa ggtctgggac cagtcttcag tataaatctc agccagaaaa 6180
accaactcct catcttgatc tgcaggaaaa caccaaacac actatggaac tctgctgatg 6240
gggacccaag cgcccacgtg ctcagccacc ctctggctca gcggggccca gacccacctc 6300
ggcacggaca cccctgtctc caggaggggc aggtggctga ggctcttcgg agctgtcagc 6360
gcccggtgcc tgccctgggc acctccctgc agtcatctct ttgcactttg ttactctttc 6420
aaagcattca caaacttttg tacctagctc tagcctgtac cagttagttc atcaaaggaa 6480
accaaccggg atgctaacaa caacatggtt agaatcctaa ttagctactt taagatccta 6540
ggattggttg gtttttcttt ttttttttctc tttgtttctt tccttttttt ttttttttt 6600
taagacaaca gaattcttaa tagatttgaa tagcgacgta tttcctgttg tagtcatttt 6660
tagctcgacc acatcatcag gtctttgcca ccgaggcata gtgtagaaca gtcccggtca 6720
gttggccaac ctcccgcagc caagtaggtt catccttgtt cctgttcatt ctcatagatg 6780

Fig. 4 (continued)

gccctgcttt ccccagggtg acatcgtagc caaatgttta ctgttttcat tgccttttat 6840
ggccttgacg acttcccctc ccaccagctg agaatgtatg gaggtcatcg gggcctcagc 6900
tcggaggcag tgacttgggg ccaagggacc tcgagacgct ttccttcccc acccccagc 6960
gtcatctccc cagcctgctg ttcccgcttt ccatatagct ttggccagga aagcatgcaa 7020
tagacttgct cggagcccag cactcctggg tctcggggtc ggggagggga cgggggcacc 7080
cacttccttg tctgtgacgg cgtgttgttc cccactctgg gatggggaag aggcccgtcg 7140
ggagttctgc atggcagttc actgcatgtg ctgccccctt gggttgctct gccaatgtat 7200
taataccatc ccatagctcc tgccaaatcg agaccctctg acgacttgcc gactaactgg 7260
ccaccacaag ctgcagtctg tagcactgaa caaacaaaaa acaaaacgct caagccttac 7320
gaccagagaa ggatttcagc aaaccaccac ctcccactca gtgtcccctc caaacttcac 7380
acttccctgc ctgcagagga tgactctgtt cacacccaat ccagcgcggt tctaccccac 7440
gaaactgtga ctttccaaat gagcctttcc ctagggctag acctaagacc aggaagtttg 7500
agaaagcagc cgcagctcaa ctcttccagc tccgccaggg ttgggaagtc cttaggtgca 7560
gtgcggctcc cactgggtct gcggaccctc ctattagagt acgaaattcc tggcaactgg 7620
tatagaacca acctagaggc tttgcagttg gcaagctaac tcgcggcctt atttctgcct 7680
ttaatctccc acaaggcatc tgttgctttg ggtcctccac gactcttagg cccgcctcaa 7740
caacccaggc acctcctagg taggctcaaa ggtagacccg tttccaccgc agcaggtgaa 7800
catgaccgtg ttttcaactg tgtccacagt tcagatccct ttccagattg caacctggcc 7860
tgcatcccag ctccttcctg ctcgtgtctt aacctaagtg ctttcttgtt tgaaacgcct 7920
acaaacctcc atgtggtagc tcctttggca aatgtcctgc tgtggcgttt tatgtgttgc 7980
ttggagtctg tggggtcgta ctccctcccc tcccgtcccc agggcagatt tgattgaatg 8040
tttgctgaag ttttgtctct tggtccacag tatttggaaa ggtcactgaa aatgggtctt 8100
tcagtcttgg catttcattt aggatctcca tgagaaatgg gcttcttgag ccctgaaaat 8160
gtatattgtg tgtctcatct gtgaactgct ttctgctata tagaactagc tcaaaagact 8220
gtacatattt acaagaaact ttatattcgt aaaaaaaaaa agaggaaatt gaattggttt 8280
ctacttttttt attgtaaaag gtgcattttt caacacttac ttttggtttc aatggtggta 8340
gttgtggaca gccatcttca ctggagggtg gggagctccg tgtgaccacc aagatgccag 8400
caggatatac cgtaacacga aattgctgtc aaaagcttat tagcatcaat caagattcta 8460
ggtctccaaa agtacaggct ttttcttcat tacctttttt attcagaacg aggaagagaa 8520
cacaaggaat gattcaagat ccaccttgag aggaatgaac tttgttgttg aacaattagt 8580
gaaataaagc aatgatctaa act 8603

Fig. 5

```
Val Leu Asp Asn Gln Ile Lys Lys Asp Leu Ala Asp Lys Glu Thr Leu
 1               5                  10                  15
Glu Asn Met Met Gln Arg His Glu Glu Glu Ala His Glu Lys Gly Lys
            20                  25                  30
Ile Leu Ser Glu Gln Lys Ala Met Ile Asn Ala Met Asp Ser Lys Ile
        35                  40                  45
Arg Ser Leu Glu Gln Arg Ile Val Glu Leu Ser Glu Ala Asn Lys Leu
    50                  55                  60
Ala Ala Asn Ser Ser Leu Phe Thr Gln Arg Asn Met Lys Ala Gln Glu
65                  70                  75                  80
Glu Met Ile Ser Glu Leu Arg Gln Gln Lys Phe Tyr Leu Glu Thr Gln
                85                  90                  95
Ala Gly Lys Leu Glu Ala Gln Asn Arg Lys Leu Glu Glu Gln Leu Glu
            100                 105                 110
Lys Ile Ser His Gln Asp His Ser Asp Lys Asn Arg Leu Leu Glu Leu
        115                 120                 125
Glu Thr Arg Leu Arg Glu Val Ser Leu Glu His Glu Glu Gln Lys Leu
    130                 135                 140
Glu Leu Lys Arg Gln Leu Thr Glu Leu Gln Leu Ser Leu Gln Glu Arg
145                 150                 155                 160
Glu Ser Gln Leu Thr Ala Leu Gln Ala Ala Arg Ala Ala Leu Glu Ser
                165                 170                 175
Gln Leu Arg Gln Ala Lys Thr Glu Leu Glu Glu Thr Thr Ala Glu Ala
            180                 185                 190
Glu Glu Glu Ile Gln Ala Leu Thr Ala His Arg Asp Glu Ile Gln Arg
        195                 200                 205
Lys Phe Asp Ala Leu Arg Asn Ser Cys Thr Val Ile Thr Asp Leu Glu
    210                 215                 220
Glu Gln Leu Asn Gln Leu Thr Glu Asp Asn Ala Glu Leu Asn Asn Gln
225                 230                 235                 240
Asn Phe Tyr Leu Ser Lys Gln Leu Asp Glu Ala Ser Gly Ala Asn Asp
                245                 250                 255
Glu Ile Val Gln Leu Arg Ser Glu Val Asp His Leu Arg Arg Glu Ile
            260                 265                 270
Thr Glu Arg Glu Met Gln Leu Thr Ser Gln Lys Gln Thr Met Glu Ala
        275                 280                 285
Leu Lys Thr Thr Cys Thr Met Leu Glu Glu Gln Val Met Asp Leu Glu
    290                 295                 300
```

Fig. 5 (continued)

```
Ala Leu Asn Asp Glu Leu Leu Glu Lys Glu Arg Gln Trp Glu Ala Trp
305                 310                 315                 320
Arg Ser Val Leu Gly Asp Glu Lys Ser Gln Phe Glu Cys Arg Val Arg
                325                 330                 335
Glu Leu Gln Arg Met Leu Asp Thr Glu Lys Gln Ser Arg Ala Arg Ala
            340                 345                 350
Asp Gln Arg Ile Thr Glu Ser Arg Gln Val Val Glu Leu Ala Val Lys
        355                 360                 365
Glu His Lys Ala Glu Ile Leu Ala Leu Gln Gln Ala Leu Lys Glu Gln
    370                 375                 380
Lys Leu Lys Ala Glu Ser Leu Ser Asp Lys Leu Asn Asp Leu Glu Lys
385                 390                 395                 400
Lys His Ala Met Leu Glu Met Asn Ala Arg Ser Leu Gln Gln Lys Leu
                405                 410                 415
Glu Thr Glu Arg Glu Leu Lys Gln Arg Leu Leu Glu Glu Gln Ala Lys
            420                 425                 430
Leu Gln Gln Gln Met Asp Leu Gln Lys Asn His Ile Phe Arg Leu Thr
        435                 440                 445
Gln Gly Leu Gln Glu Ala Leu Asp Arg Ala Asp Leu Leu Lys Thr Glu
    450                 455                 460
Arg Ser Asp Leu Glu Tyr Gln Leu Glu Asn Ile Gln Val Leu Tyr Ser
465                 470                 475                 480
His Glu Lys Val Lys Met Glu Gly Thr Ile Ser Gln Gln Thr Lys Leu
                485                 490                 495
Ile Asp Phe Leu Gln Ala Lys Met Asp Gln Pro Ala Lys Lys Lys Lys
            500                 505                 510
Val Pro Leu Gln Tyr Asn Glu Leu Lys Leu Ala Leu Glu Lys Glu Lys
        515                 520                 525
Ala Arg Cys Ala Glu Leu Glu Glu Ala Leu Gln Lys Thr Arg Ile Glu
    530                 535                 540
Leu Arg Ser Ala Arg Glu Glu Ala Ala His Arg Lys Ala Thr Asp His
545                 550                 555                 560
Pro His Pro Ser Thr Pro Ala Thr Ala Arg Gln Gln Ile Ala Met Ser
                565                 570                 575
Ala Ile Val Arg Ser Pro Glu His Gln Pro Ser Ala Met Ser Leu Leu
            580                 585                 590
Ala Pro Pro Ser Ser Arg Arg Lys Glu Ser Ser Thr Pro Glu Glu Phe
        595                 600                 605
```

Fig. 5 (continued)

```
Ser Arg Arg Leu Lys Glu Arg Met His His Asn Ile Pro His Arg Phe
    610                 615                 620
Asn Val Gly Leu Asn Met Arg Ala Thr Lys Cys Ala Val Cys Leu Asp
625                 630                 635                 640
Thr Val His Phe Gly Arg Gln Ala Ser Lys Cys Leu Glu Cys Gln Val
                645                 650                 655
Met Cys His Pro Lys Cys Ser Thr Cys Leu Pro Ala Thr Cys Gly Leu
            660                 665                 670
Pro Ala Glu Tyr Ala Thr His Phe Thr Glu Ala Phe Cys Arg Asp Lys
        675                 680                 685
Met Asn Ser Pro Gly Leu Gln Thr Lys Glu Pro Ser Ser Ser Leu His
    690                 695                 700
Leu Glu Gly Trp Met Lys Val Pro Arg Asn Asn Lys Arg Gly Gln Gln
705                 710                 715                 720
Gly Trp Asp Arg Lys Tyr Ile Val Leu Glu Gly Ser Lys Val Leu Ile
                725                 730                 735
Tyr Asp Asn Glu Ala Arg Glu Ala Gly Gln Arg Pro Val Glu Glu Phe
            740                 745                 750
Glu Leu Cys Leu Pro Asp Gly Asp Val Ser Ile His Gly Ala Val Gly
        755                 760                 765
Ala Ser Glu Leu Ala Asn Thr Ala Lys Ala Asp Val Pro Tyr Ile Leu
    770                 775                 780
Lys Met Glu Ser His Pro His Thr Thr Cys Trp Pro Gly Arg Thr Leu
785                 790                 795                 800
Tyr Leu Leu Ala Pro Ser Phe Pro Asp Lys Gln Arg Trp Val Thr Ala
                805                 810                 815
Leu Glu Ser Val Val Ala Gly Gly Arg Val Ser Arg Glu Lys Ala Glu
            820                 825                 830
Ala Asp Ala Lys Leu Leu Gly Asn Ser Leu Leu Lys Leu Glu Gly Asp
        835                 840                 845
Asp Arg Leu Asp Met Asn Cys Thr Leu Pro Phe Ser Asp Gln Val Val
    850                 855                 860
Leu Val Gly Thr Glu Glu Gly Leu Tyr Ala Leu Asn Val Leu Lys Asn
865                 870                 875                 880
Ser Leu Thr His Val Pro Gly Ile Gly Ala Val Phe Gln Ile Tyr Ile
                885                 890                 895
Ile Lys Asp Leu Glu Lys Leu Leu Met Ile Ala Gly Glu Glu Arg Ala
            900                 905                 910
```

Fig. 5 (continued)

```
Leu Cys Leu Val Asp Val Lys Lys Val Lys Gln Ser Leu Ala Gln Ser
        915                 920                 925
His Leu Pro Ala Gln Pro Asp Ile Ser Pro Asn Ile Phe Glu Ala Val
    930                 935                 940
Lys Gly Cys His Leu Phe Gly Ala Gly Lys Ile Glu Asn Gly Leu Cys
945                 950                 955                 960
Ile Cys Ala Ala Met Pro Ser Lys Val Val Ile Leu Arg Tyr Asn Glu
                965                 970                 975
Asn Leu Ser Lys Tyr Cys Ile Arg Lys Glu Ile Glu Thr Ser Glu Pro
            980                 985                 990
Cys Ser Cys Ile His Phe Thr Asn Tyr Ser Ile Leu Ile Gly Thr Asn
        995                 1000                1005
Lys Phe Tyr Glu Ile Asp Met Lys Gln Tyr Thr Leu Glu Glu Phe Leu
    1010                1015                1020
Asp Lys Asn Asp His Ser Leu Ala Pro Ala Val Phe Ala Ala Ser Ser
1025                1030                1035                1040
Asn Ser Phe Pro Val Ser Ile Val Gln Val Asn Ser Ala Gly Gln Arg
                1045                1050                1055
Glu Glu Tyr Leu Leu Cys Phe His Glu Phe Gly Val Phe Val Asp Ser
            1060                1065                1070
Tyr Gly Arg Arg Ser Arg Thr Asp Asp Leu Lys Trp Ser Arg Leu Pro
        1075                1080                1085
Leu Ala Phe Ala Tyr Arg Glu Pro Tyr Leu Phe Val Thr His Phe Asn
    1090                1095                1100
Ser Leu Glu Val Ile Glu Ile Gln Ala Arg Ser Ser Ala Gly Thr Pro
1105                1110                1115                1120
Ala Arg Ala Tyr Leu Asp Ile Pro Asn Pro Arg Tyr Leu Gly Pro Ala
                1125                1130                1135
Ile Ser Ser Gly Ala Ile Tyr Leu Ala Ser Ser Tyr Gln Asp Lys Leu
            1140                1145                1150
Arg Val Ile Cys Cys Lys Gly Asn Leu Val Lys Glu Ser Gly Thr Glu
        1155                1160                1165
His His Arg Gly Pro Ser Thr Ser Arg Ser Ser Pro Asn Lys Arg Gly
    1170                1175                1180
Pro Pro Thr Tyr Asn Glu His Ile Thr Lys Arg Val Ala Ser Ser Pro
1185                1190                1195                1200
Ala Pro Pro Glu Gly Pro Ser His Pro Arg Glu Pro Ser Thr Pro His
                1205                1210                1215
```

Fig. 5 (continued)

```
Arg Tyr Arg Glu Gly Arg Thr Glu Leu Arg Arg Asp Lys Ser Pro Gly
            1220                1225                1230
Arg Pro Leu Glu Arg Glu Lys Ser Pro Gly Arg Met Leu Ser Thr Arg
        1235                1240                1245
Arg Glu Arg Ser Pro Gly Arg Leu Phe Glu Asp Ser Ser Arg Gly Arg
    1250                1255                1260
Leu Pro Ala Gly Ala Val Arg Thr Pro Leu Ser Gln Val Asn Lys Val
1265                1270                1275                1280
Trp Asp Gln Ser Ser Val
                1285
```

Fig. 6

```
cagagcaggg cgagagccga tcagcggatc accgagtctc gccaggtggt ggagctggca      60
gtgaaggagc acaaggctga gattctcgct ctgcagcagg ctctcaaaga gcagaagctg     120
aaggccgaga gcctctctga caagctcaat gacctggaga agaagcatgc tatgcttgaa     180
atgaatgccc gaagcttaca gcagaagctg gagactgaac gagagctcaa acagaggctt     240
ctggaagagc aagccaaatt acagcagcag atggacctgc agaaaaatca cattttccgt     300
ctgactcaag gactgcaaga agctctagat cgggctgatc tactgaagac agaaagaagt     360
gacttggagt atcagctgga aaacattcag gttctctatt ctcatgaaaa ggtgaaaatg     420
gaaggcacta tttctcaaca aaccaaactc attgattttc tgcaagccaa aatggaccaa     480
cctgctaaaa agaaaaaggt tcctctgcag tacaatgagc tgaagctggc cctggagaag     540
gagaaagctc gctgtgcaga gctagaggaa gcccttcaga agacccgcat cgagctccgg     600
tccgcccggg aggaagctgc ccaccgcaaa gcaacggacc acccacaccc atccacgcca     660
gccaccgcga ggcagcagat cgccatgtct gccatcgtgc ggtcgccaga gcaccagccc     720
agtgccatga gcctgctggc cccgccatcc agccgcagaa aggagtcttc aactccagag     780
gaatttagtc ggcgtcttaa ggaacgcatg caccacaata ttcctcaccg attcaacgta     840
ggactgaaca tgcgagccac aaagtgtgct gtgtgtctgg ataccgtgca ctttggacgc     900
caggcatcca aatgtctcga atgtcaggtg atgtgtcacc ccaagtgctc cacgtgcttg     960
ccagccacct gcggcttgcc tgctgaatat gccacacact tcaccgaggc cttctgccgt    1020
gacaaaatga actccccagg tctccagacc aaggagccca gcagcagctt gcacctggaa    1080
gggtggatga aggtgcccag gaataacaaa cgaggacagc aaggctggga caggaagtac    1140
attgtcctgg agggatcaaa agtcctcatt tatgacaatg aagccagaga agctggacag    1200
aggccggtgg aagaatttga gctgtgcctt cccgacgggg atgtatctat tcatggtgcc    1260
gttggtgctt ccgaactcgc aaatacagcc aaagcagatg tcccatacat actgaagatg    1320
gaatctcacc cgcacaccac ctgctggccc gggagaaccc tctacttgct agctcccagc    1380
ttccctgaca aacagcgctg ggtcaccgcc ttagaatcag ttgtcgcagg tgggagagtt    1440
tctagggaaa aagcagaagc tgatgctaaa ctgcttggaa actccctgct gaaactggaa    1500
ggtgatgacc gtctagacat gaactgcacg ctgcccttca gtgaccaggt ggtgttggtg    1560
ggcaccgagg aagggctcta cgccctgaat gtcttgaaaa actccctaac ccatgtccca    1620
ggaattggag cagtcttcca aatttatatt atcaaggacc tggagaagct actcatgata    1680
gcaggagaag agcgggcact gtgtcttgtg gacgtgaaga aagtgaaaca gtccctggcc    1740
cagtcccacc tgcctgccca gcccgacatc tcacccaaca tttttgaagc tgtcaagggc    1800
tgccacttgt ttggggcagg caagattgag aacgggctct gcatctgtgc agccatgccc    1860
agcaaagtcg tcattctccg ctacaacgaa aacctcagca aatactgcat ccggaaagag    1920
atagagacct cagagccctg cagctgtatc cacttcacca attacagtat cctcattgga    1980
accaataaat tctacgaaat cgacatgaag cagtacacgc tcgaggaatt cctggataag    2040
aatgaccatt ccttggcacc tgctgtgttt gccgcctctt ccaacagctt ccctgtctca    2100
atcgtgcagg tgaacagcgc agggcagcga gaggagtact tgctgtgttt ccacgaattt    2160
ggagtgttcg tggattctta cggaagacgt agccgcacag acgatctcaa gtggagtcgc    2220
ttacctttgg cctttgccta cagagaaccc tatctgtttg tgacccactt caactcactc    2280
```

Fig. 6 (continued)

gaagtaattg agatccaggc acgctcctca gcagggaccc ctgcccgagc gtacctggac 2340
atcccgaacc cgcgctacct gggccctgcc atttcctcag gagcgattta cttggcgtcc 2400
tcataccagg ataaattaag ggtcatttgc tgcaagggaa acctcgtgaa ggagtccggc 2460
actgaacacc accggggccc gtccacctcc cgcagcagcc ccaacaagcg aggcccaccc 2520
acgtacaacg agcacatcac caagcgcgtg gcctccagcc cagcgccgcc cgaaggcccc 2580
agccacccgc gagagccaag cacaccccac cgctaccgcg aggggcggac cgagctgcgc 2640
agggacaagt ctcctggccg ccccctggag cgagagaagt cccccggccg gatgctcagc 2700
acgcggagag agcggtcccc cgggaggctg tttgaagaca gcagcagggg ccggctgcct 2760
gcgggagccg tgaggacccc gctgtcccag gtgaacaagg tctgggacca gtcttcagta 2820
taaatctcag ccagaaaaac caactcctca tcttgatctg caggaaaaca ccaaacacac 2880
tatggaactc tgctgatggg gacccaagcg cccacgtgct cagccaccct ctggctcagc 2940
ggggcccaga cccacctcgg cacggacacc cctgtctcca ggaggggcag gtggctgagg 3000
ctcttcggag ctgtcagcgc ccggtgcctg ccctgggcac ctccctgcag tcatctcttt 3060
gcactttgtt actctttcaa agcattcaca aacttttgta cctagctcta gcctgtacca 3120
gttagttcat caaaggaaac caaccgggat gctaacaaca acatggttag aatcctaatt 3180
agctacttta agatcctagg attggttggt ttttcttttt ttttttctctt tgtttctttc 3240
ctttttttt ttttttttta agacaacaga attcttaata gatttgaata gcgacgtatt 3300
tcctgttgta gtcatttta gctcgaccac atcatcaggt ctttgccacc gaggcatagt 3360
gtagaacagt cccggtcagt tggccaacct cccgcagcca agtaggttca tccttgttcc 3420
tgttcattct catagatggc cctgctttcc ccagggtgac atcgtagcca aatgtttact 3480
gttttcattg ccttttatgg ccttgacgac ttcccctccc accagctgag aatgtatgga 3540
ggtcatcggg gcctcagctc ggaggcagtg acttggggcc aagggacctc gagacgcttt 3600
ccttccccac cccccagcgt catctcccca gcctgctgtt cccgctttcc atatagcttt 3660
ggccaggaaa gcatgcaata gacttgctcg gagcccagca ctcctgggtc tcggggtcgg 3720
ggaggggacg ggggcaccca cttccttgtc tgtgacggcg tgttgttccc cactctggga 3780
tggggaagag gcccgtcggg agttctgcat ggcagttcac tgcatgtgct gccccccttgg 3840
gttgctctgc caatgtatta ataccatccc atagctcctg ccaaatcgag accctctgac 3900
gacttgccga ctaactggcc accacaagct gcagtctgta gcactgaaca aacaaaaaac 3960
aaaacgctca agccttacga ccagagaagg atttcagcaa accaccacct cccactcagt 4020
gtcccctcca aacttcacac ttccctgcct gcagaggatg actctgttca cacccaatcc 4080
agcgcggttc taccccacga aactgtgact ttccaaatga gcctttccct agggctagac 4140
ctaagaccag gaagtttgag aaagcagccg cagctcaact cttccagctc cgccagggtt 4200
gggaagtcct taggtgcagt gcggctccca ctgggtctgc ggaccctcct attagagtac 4260
gaaattcctg gcaactggta tagaaccaac ctagaggctt tgcagttggc aagctaactc 4320
gcggccttat ttctgccttt aatctcccac aaggcatctg ttgctttggg tcctccacga 4380
ctcttaggcc cgcctcaaca acccaggcac ctcctaggta ggctcaaagg tagacccgtt 4440
tccaccgcag caggtgaaca tgaccgtgtt ttcaactgtg tccacagttc agatcccttt 4500
ccagattgca acctggcctg catcccagct ccttcctgct cgtgtcttaa cctaagtgct 4560
ttcttgtttg aaacgcctac aaacctccat gtggtagctc ctttggcaaa tgtcctgctg 4620

Fig. 6 (continued)

```
tggcgtttta tgtgttgctt ggagtctgtg gggtcgtact ccctcccctc ccgtccccag    4680
ggcagatttg attgaatgtt tgctgaagtt ttgtctcttg gtccacagta tttggaaagg    4740
tcactgaaaa tgggtctttc agtcttggca tttcatttag gatctccatg agaaatgggc    4800
ttcttgagcc ctgaaaatgt atattgtgtg tctcatctgt gaactgcttt ctgctatata    4860
gaactagctc aaaagactgt acatatttac aagaaacttt atattcgtaa aaaaaaaaag    4920
aggaaattga attggtttct actttttat tgtaaaaggt gcatttttca acacttactt    4980
ttggtttcaa tggtggtagt tgtggacagc catcttcact ggagggtggg gagctccgtg    5040
tgaccaccaa gatgccagca ggatataccg taacacgaaa ttgctgtcaa aagcttatta    5100
gcatcaatca agattctagg tctccaaaag tacaggcttt ttcttcatta cctttttat     5160
tcagaacgag gaagagaaca caaggaatga ttcaagatcc accttgagag gaatgaactt    5220
tgttgttgaa caattagtga aataaagcaa tgatctaaac t                       5261
```

Fig. 7

```
Met Ser Ala Glu Val Arg Leu Arg Gln Leu Gln Gln Leu Val Leu Asp
 1               5                  10                  15
Pro Gly Phe Leu Gly Leu Glu Pro Leu Leu Asp Leu Leu Leu Gly Val
            20                  25                  30
His Gln Glu Leu Gly Ala Ser His Leu Ala Gln Asp Lys Tyr Val Ala
        35                  40                  45
Asp Phe Leu Gln Trp Val Glu Pro Ile Ala Ala Arg Leu Lys Glu Val
    50                  55                  60
Arg Leu Gln Arg Asp Asp Phe Glu Ile Leu Lys Val Ile Gly Arg Gly
65                  70                  75                  80
Ala Phe Ser Glu Val Ala Val Val Lys Met Lys Gln Thr Gly Gln Val
                85                  90                  95
Tyr Ala Met Lys Ile Met Asn Lys Trp Asp Met Leu Lys Arg Gly Glu
            100                 105                 110
Val Ser Cys Phe Arg Glu Glu Arg Asp Val Leu Val Lys Gly Asp Arg
        115                 120                 125
Arg Trp Ile Thr Gln Leu His Phe Ala Phe Gln Asp Glu Asn Tyr Leu
    130                 135                 140
Tyr Leu Val Met Glu Tyr Tyr Val Gly Gly Asp Leu Leu Thr Leu Leu
145                 150                 155                 160
Ser Lys Phe Gly Glu Arg Ile Pro Ala Glu Met Ala Arg Phe Tyr Leu
                165                 170                 175
Ala Glu Ile Val Met Ala Ile Asp Ser Val His Arg Leu Gly Tyr Val
            180                 185                 190
His Arg Asp Ile Lys Pro Asp Asn Ile Leu Leu Asp Arg Cys Gly His
        195                 200                 205
Ile Arg Leu Ala Asp Phe Gly Ser Cys Leu Lys Leu Gln Pro Asp Gly
    210                 215                 220
Met Val Arg Ser Leu Val Ala Val Gly Thr Pro Asp Tyr Leu Ser Pro
225                 230                 235                 240
Glu Ile Leu Gln Ala Val Gly Gly Gly Pro Gly Ala Gly Ser Tyr Gly
                245                 250                 255
Pro Glu Cys Asp Trp Trp Ala Leu Gly Val Phe Ala Tyr Glu Met Phe
            260                 265                 270
Tyr Gly Gln Thr Pro Phe Tyr Ala Asp Ser Thr Ala Glu Thr Tyr Ala
        275                 280                 285
```

Fig. 7 (continued)

```
Lys Ile Val His Tyr Arg Glu His Leu Ser Leu Pro Leu Ala Asp Thr
    290                 295                 300
Val Val Pro Glu Glu Ala Gln Asp Leu Ile Arg Gly Leu Leu Cys Pro
305                 310                 315                 320
Ala Glu Ile Arg Leu Gly Arg Gly Gly Ala Gly Asp Phe Gln Lys His
                325                 330                 335
Pro Phe Phe Phe Gly Leu Asp Trp Glu Gly Leu Arg Asp Ser Val Pro
            340                 345                 350
Pro Phe Thr Pro Asp Phe Glu Gly Ala Thr Asp Thr Cys Asn Phe Asp
        355                 360                 365
Val Val Glu Asp Arg Leu Thr Ala Met Val Ser Gly Gly Gly Glu Thr
    370                 375                 380
Leu Ser Asp Met Gln Glu Asp Met Pro Leu Gly Val Arg Leu Pro Phe
385                 390                 395                 400
Val Gly Tyr Ser Tyr Cys Cys Met Ala Phe Arg Asp Asn Gln Val Pro
                405                 410                 415
Asp Pro Thr Pro Met Glu Leu Glu Ala Leu Gln Leu Pro Val Ser Asp
            420                 425                 430
Leu Gln Gly Leu Asp Leu Gln Pro Pro Val Ser Pro Pro Asp Gln Val
        435                 440                 445
Ala Glu Glu Ala Asp Leu Val Ala Val Pro Ala Pro Val Ala Glu Ala
    450                 455                 460
Glu Thr Thr Val Thr Leu Gln Gln Leu Gln Glu Ala Leu Glu Glu Glu
465                 470                 475                 480
Val Leu Thr Arg Gln Ser Leu Ser Arg Glu Leu Glu Ala Ile Arg Thr
                485                 490                 495
Ala Asn Gln Asn Phe Ser Ser Gln Leu Gln Glu Ala Glu Val Arg Asn
            500                 505                 510
Arg Asp Leu Glu Ala His Val Arg Gln Leu Gln Glu Arg Met Glu Met
        515                 520                 525
Leu Gln Ala Pro Gly Ala Ala Ala Ile Thr Gly Val Pro Ser Pro Arg
    530                 535                 540
Ala Thr Asp Pro Pro Ser His Leu Asp Gly Pro Pro Ala Val Ala Val
545                 550                 555                 560
Gly Gln Cys Pro Leu Val Gly Pro Gly Pro Met His Arg Arg His Leu
                565                 570                 575
Leu Leu Pro Ala Arg Ile Pro Arg Pro Gly Leu Ser Glu Ala Arg Cys
            580                 585                 590
```

Fig. 7 (continued)

```
Leu Leu Leu Phe Ala Ala Ala Leu Ala Ala Ala Ala Thr Leu Gly Cys
        595                 600                 605
Thr Gly Leu Val Ala Tyr Thr Gly Gly Leu Thr Pro Val Trp Cys Phe
    610                 615                 620
Pro Gly Ala Thr Phe Ala Pro
625                 630
```

Fig. 8

BLASTP - alignment of 543_Protein against trembl|AF086824|AF086824_1 gene: "Crik"; product: "rho/rac-interacting citron kinase"; Mus musculus rho/rac-interacting citron kinase (Crik) mRNA, complete cds. //:gp|AF086824|3599509 gene: "Crik"; product: "rho/rac-interacting citron kinase"; Mus musculus rho/rac-interacting citron kinase (Crik) mRNA, complete cds.

This hit is scoring at : 0.0 (expectation value)
Alignment length (overlap) : 2056
Identities : 96 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb_1_;

```
Q:      1 MLKFKYGARNPLDAGAAEPIASRASRLNLFFQGKPPFMTQQQMSPLSREGILDALFVLFE
          MLKFKYG.RNP :A.A:EPIASRASRLNLFFQGKPP.MTQQQMS.LSREG:LDALF.LFE
H:      1 MLKFKYGVRNPPEASASEPIASRASRLNLFFQGKPPLMTQQQMSALSREGMLDALFALFE

                                    Protein_Kinase_ATP Motif (K binds ATP)
          ECSQPALMKIKHVSNFVRKYSDTIAELQELQPSAKDFEVRSLVGCGHFAEVQVVREKATG
          ECSQPALMK:KHVS:FV:KYSDTIAEL:ELQPSA:DFEVRSLVGCGHFAEVQVVREKATG
          ECSQPALMKMKHVSSFVQKYSDTIAELRELQPSARDFEVRSLVGCGHFAEVQVVREKATG

          DIYAMKVMKKKALLAQEQVSFFEEERNILSRSTSPWIPQLQYAFQDKNHLYLVMEYQPGG
          D:YAMK:MKKKALLAQEQVSFFEEERNILSRSTSPWIPQLQYAFQDKN:LYLVMEYQPGG
          DVYAMKIMKKKALLAQEQVSFFEEERNILSRSTSPWIPQLQYAFQDKNNLYLVMEYQPGG

                             Protein_Kinase_ST Motif (D is an active site)
          DLLSLLNRYEDQLDENLIQFYLAELILAVHSVHLMGYVHRDIKPENILVDRTGHIKLVDF
          D.LSLLNRYEDQLDE::IQFYLAELILAVHSVH MGYVHRDIKPENIL:DRTG.IKLVDF
          DFLSLLNRYEDQLDESMIQFYLAELILAVHSVHQMGYVHRDIKPENILIDRTGEIKLVDF

          GSAAKMNSNKMVNAKLPIGTPDYMAPEVLTVMNGDGKGTYGLDCDWWSVGVIAYEMIYGR
          GSAAKMNSNK V:AKLPIGTPDYMAPEVLTVMN D :GTYGLDCDWWSVGV:AYEM:YG:
          GSAAKMNSNK-VDAKLPIGTPDYMAPEVLTVMNEDRRGTYGLDCDWWSVGVVAYEMVYGK

          SPFAEGTSARTFNNIMNFQRFLKFPDDPKVSSDFLDLIQSLLCGQKERLKFEGLCCHPFF
          :PF.EGTSARTFNNIMNFQRFLKFPDDPKVSS:.LDL:QSLLC QKERLKFEGLCCHPFF
          TPFTEGTSARTFNNIMNFQRFLKFPDDPKVSSELLDLLQSLLCVQKERLKFEGLCCHPFF
```

Fig. 8 (continued)

```
SKIDWNNIRNSPPPFVPTLKSDDDTSNFDEPEKNSWVSSSPCQLSPSGFSGEELPFVGFS
::.DWNNIRNSPPPFVPTLKSDDDTSNFDEPEKNSW.    ....P .FSGEELPFVGFS
ARTDWNNIRNSPPPFVPTLKSDDDTSNFDEPEKNSWAFILCVPAEPLAFSGEELPFVGFS

YSKALGILGRSESVVSGLDSPAKTSSMEKKLLIKSKELQDSQDKCHKMEQEMTRLHRRVS
YSKALG.LGRSESVVS.LDSPAK.SSMEKKLLIKSKELQDSQDKCHKMEQEMTRLHRRVS
YSKALGYLGRSESVVSSLDSPAKVSSMEKKLLIKSKELQDSQDKCHKMEQEMTRLHRRVS

EVEAVLSQKEVELKASETQRSLLEQDLATYITECSSLKRSLEQARMEVSQEDDKALQLLH
EVEAVLSQKEVELKASETQRSLLEQDLATYITECSSLKRSLEQARMEVSQEDDKALQLLH
EVEAVLSQKEVELKASETQRSLLEQDLATYITECSSLKRSLEQARMEVSQEDDKALQLLH

DIREQSRKLQEIKEQEYQAQVEEMRLMMNQLEEDLVSARRRSDLYESELRESRLAAEEFK
DIREQSRKLQEIKEQEYQAQVEEMRLMMNQLEEDLVSARRRSDLYESELRESRLAAEEFK
DIREQSRKLQEIKEQEYQAQVEEMRLMMNQLEEDLVSARRRSDLYESELRESRLAAEEFK

RKATECQHKLLKAKDQGKPEVGEYAKLEKINAEQQLKIQELQEKLEKAVKASTEATELLQ
RKA.ECQHKL:KAKDQGKPEVGEY:KLEKINAEQQLKIQELQEKLEKAVKASTEATELLQ
RKANECQHKLMKAKDQGKPEVGEYSKLEKINAEQQLKIQELQEKLEKAVKASTEATELLQ

NIRQAKERAERELEKLQNREDSSEGIRKKLVEAEERRHSLENKVKRLETMERRENRLKDD
NIRQAKERAERELEKL.NREDSSEGI:KKLVEAEERRHSLENKVKRLETMERRENRLKDD
NIRQAKERAERELEKLHNREDSSEGIKKKLVEAEERRHSLENKVKRLETMERRENRLKDD

IQTKSQQIQQMADKILELEEKHREAQVSAQHLEVHLKQKEQHYEEKIKVLDNQIKKDLAD
IQTKS:QIQQMADKILELEEKHREAQVSAQHLEVHLKQKEQHYEEKIKVLDNQIKKDLAD
IQTKSEQIQQMADKILELEEKHREAQVSAQHLEVHLKQKEQHYEEKIKVLDNQIKKDLAD

KETLENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSEANKLAANSSLFT
KE:LENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSEANKLAANSSLFT
KESLENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSEANKLAANSSLFT

QRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHSDKNRLLELETRL
QRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHSDK:RLLELETRL
QRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHSDKSRLLELETRL

REVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQAKTELEETTAEA
REVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQAKTELEETTAEA
REVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQAKTELEETTAEA
```

Fig. 8 (continued)

EEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQNFYLSKQLDEAS
EEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQNFYLSKQLDEAS
EEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQNFYLSKQLDEAS

GANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQVMDLEALNDELLE
GANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQV:DLEALNDELLE
GANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQVLDLEALNDELLE

KERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESRQVVELAVKEHKA
KERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESRQVVELAVKEHKA
KERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESRQVVELAVKEHKA

EILALQQALKEQKLKAESLSDKLNDLEKKHAMLEMNARSLQQKLETERELKQRLLEEQAK
EILALQQALKEQKLKAESLSDKLNDLEKKHAMLEMNARSLQQKLETERELKQRLLEEQAK
EILALQQALKEQKLKAESLSDKLNDLEKKHAMLEMNARSLQQKLETERELKQRLLEEQAK

LQQQMDLQKNHIFRLTQGLQEALDRADLLKTERSDLEYQLENIQVLYSHEKVKMEGTISQ
LQQQMDLQKNHIFRLTQGLQEALDRADLLKTERSDLEYQLENIQVLYSHEKVKMEGTISQ
LQQQMDLQKNHIFRLTQGLQEALDRADLLKTERSDLEYQLENIQVLYSHEKVKMEGTISQ

QTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEKEKARCAELEEALQKTRIELRSAREEA
QTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEKEKARCAELEEALQKTRIELRSAREEA
QTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEKEKARCAELEEALQKTRIELRSAREEA

AHRKATDHPHPSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRKESSTPEEFSRRL
AHRKATDHPHPSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRKESSTPEEFSRRL
AHRKATDHPHPSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRKESSTPEEFSRRL

KERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGRQASKCLECQVMCHPKCSTCLPATCGL
KERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGRQASKCLECQVMCHPKCSTCLPATCGL
KERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGRQASKCLECQVMCHPKCSTCLPATCGL

PAEYATHFTEAFCRDKMNSPGLQTKEPSSSLHLEGWMKVPRNNKRGQQGWDRKYIVLEGS
PAEYATHFTEAFCRDKMNSPGLQ:KEP.SSLHLEGWMKVPRNNKRGQQGWDRKYIVLEGS
PAEYATHFTEAFCRDKMNSPGLQSKEPGSSLHLEGWMKVPRNNKRGQQGWDRKYIVLEGS

KVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGAVGASELANTAKADVPYILKMESHPHT
KVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGAVGASELANTAKADVPYILKMESHPHT
KVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGAVGASELANTAKADVPYILKMESHPHT

Fig. 8 (continued)

```
TCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRVSREKAEADAKLLGNSLLKLEGDDRLD
TCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRVSREKAEADAKLLGNSLLKLEGDDRLD
TCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRVSREKAEADAKLLGNSLLKLEGDDRLD

MNCTLPFSDQVVLVGTEEGLYALNVLKNSLTHVPGIGAVFQIYIIKDLEKLLMIAGEERA
MNCTLPFSDQVVLVGTEEGLYALNVLKNSLTH:PGIGAVFQIYIIKDLEKLLMIAGEERA
MNCTLPFSDQVVLVGTEEGLYALNVLKNSLTHIPGIGAVFQIYIIKDLEKLLMIAGEERA

LCLVDVKKVKQSLAQSHLPAQPDISPNIFEAVKGCHLFGAGKIENGLCICAAMPSKVVIL
LCLVDVKKVKQSLAQSHLPAQPD:SPNIFEAVKGCHLF.AGKIEN.LCICAAMPSKVVIL
LCLVDVKKVKQSLAQSHLPAQPDVSPNIFEAVKGCHLFAAGKIENSLCICAAMPSKVVIL

RYNENLSKYCIRKEIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTLEEFLDKNDHSLA
RYN:NLSKYCIRKEIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTL:EFLDKNDHSLA
RYNDNLSKYCIRKEIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTLDEFLDKNDHSLA

PAVFAASSNSFPVSIVQVNSAGQREEYLLCFHEFGVFVDSYGRRSRTDDLKWSRLPLAFA
PAVFA:SSNSFPVSIVQ.NSAGQREEYLLCFHEFGVFVDSYGRRSRTDDLKWSRLPLAFA
PAVFASSSNSFPVSIVQANSAGQREEYLLCFHEFGVFVDSYGRRSRTDDLKWSRLPLAFA

YREPYLFVTHFNSLEVIEIQARSSAGTPARAYLDIPNPRYLGPAISSGAIYLASSYQDKL
YREPYLFVTHFNSLEVIEIQARSS.G:PARAYL:IPNPRYLGPAISSGAIYLASSYQDKL
YREPYLFVTHFNSLEVIEIQARSSLGSPARAYLEIPNPRYLGPAISSGAIYLASSYQDKL

RVICCKGNLVKESGTEHHRGPSTSRSSPNKRGPPTYNEHITKRVASSPAPPEGPSHPREP
RVICCKGNLVKESGTE.HR PSTSRSSPNKRGPPTYNEHITKRVASSPAPPEGPSHPREP
RVICCKGNLVKESGTEQHRVPSTSRSSPNKRGPPTYNEHITKRVASSPAPPEGPSHPREP

STPHRY--REGRTELRRDKSPGRPLEREKSPGRMLSTRRERSPGRLFEDSSRGRLPAGAV
STPHRY  REGRTELRRDKSPGRPLEREKSPGRMLSTRRERSPGRLFEDSSRGRLPAGAV
STPHRYRDREGRTELRRDKSPGRPLEREKSPGRMLSTRRERSPGRLFEDSSRGRLPAGAV

RTPLSQVNKVWDQSSV    2054
RTPLSQVNKVWDQSSV
RTPLSQVNKVWDQSSV    2055
```

Fig. 9

BLASTP - alignment of 543_Protein against swiss|O14578|CTRO_HUMAN
CITRON PROTEIN (FRAGMENT).//:trembl|AC002563|AC002563_2 gene:
"WUGSC:H_127H14.1";
Human PAC clone 127H14 from 12q, complete sequence. //:gp|AC002563|2439517
gene:
"WUGSC:H_127H14.1"; Human PAC clone 127H14 from 12q, complete sequence.

This hit is scoring at : 0.0 (expectation value)
Alignment length (overlap) : 1286
Identities : 100 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb_1_;

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Q:    769 VLDNQIKKDLADKETLENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSE
          VLDNQIKKDLADKETLENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSE
H:      1 VLDNQIKKDLADKETLENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSE

          ANKLAANSSLFTQRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHS
          ANKLAANSSLFTQRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHS
          ANKLAANSSLFTQRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHS

          DKNRLLELETRLREVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQ
          DKNRLLELETRLREVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQ
          DKNRLLELETRLREVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQ

          AKTELEETTAEAEEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQ
          AKTELEETTAEAEEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQ
          AKTELEETTAEAEEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQ

          NFYLSKQLDEASGANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQV
          NFYLSKQLDEASGANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQV
          NFYLSKQLDEASGANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQV

          MDLEALNDELLEKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESR
          MDLEALNDELLEKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESR
          MDLEALNDELLEKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESR
```

Fig. 9 (continued)

QVVELAVKEHKAEILALQQALKEQKLKAESLSDKLNDLEKKHAMLEMNARSLQQKLETER
QVVELAVKEHKAEILALQQALKEQKLKAESLSDKLNDLEKKHAMLEMNARSLQQKLETER
QVVELAVKEHKAEILALQQALKEQKLKAESLSDKLNDLEKKHAMLEMNARSLQQKLETER

ELKQRLLEEQAKLQQQMDLQKNHIFRLTQGLQEALDRADLLKTERSDLEYQLENIQVLYS
ELKQRLLEEQAKLQQQMDLQKNHIFRLTQGLQEALDRADLLKTERSDLEYQLENIQVLYS
ELKQRLLEEQAKLQQQMDLQKNHIFRLTQGLQEALDRADLLKTERSDLEYQLENIQVLYS

HEKVKMEGTISQQTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEKEKARCAELEEALQK
HEKVKMEGTISQQTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEKEKARCAELEEALQK
HEKVKMEGTISQQTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEKEKARCAELEEALQK

TRIELRSAREEAAHRKATDHPHPSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRK
TRIELRSAREEAAHRKATDHPHPSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRK
TRIELRSAREEAAHRKATDHPHPSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRK

ESSTPEEFSRRLKERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGRQASKCLECQVMCHP
ESSTPEEFSRRLKERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGRQASKCLECQVMCHP
ESSTPEEFSRRLKERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGRQASKCLECQVMCHP

KCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQTKEPSSSLHLEGWMKVPRNNKRGQQ
KCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQTKEPSSSLHLEGWMKVPRNNKRGQQ
KCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQTKEPSSSLHLEGWMKVPRNNKRGQQ

GWDRKYIVLEGSKVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGAVGASELANTAKADV
GWDRKYIVLEGSKVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGAVGASELANTAKADV
GWDRKYIVLEGSKVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGAVGASELANTAKADV

PYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRVSREKAEADAKLLGN
PYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRVSREKAEADAKLLGN
PYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRVSREKAEADAKLLGN

SLLKLEGDDRLDMNCTLPFSDQVVLVGTEEGLYALNVLKNSLTHVPGIGAVFQIYIIKDL
SLLKLEGDDRLDMNCTLPFSDQVVLVGTEEGLYALNVLKNSLTHVPGIGAVFQIYIIKDL
SLLKLEGDDRLDMNCTLPFSDQVVLVGTEEGLYALNVLKNSLTHVPGIGAVFQIYIIKDL

EKLLMIAGEERALCLVDVKKVKQSLAQSHLPAQPDISPNIFEAVKGCHLFGAGKIENGLC
EKLLMIAGEERALCLVDVKKVKQSLAQSHLPAQPDISPNIFEAVKGCHLFGAGKIENGLC
EKLLMIAGEERALCLVDVKKVKQSLAQSHLPAQPDISPNIFEAVKGCHLFGAGKIENGLC

Fig. 9 (continued)

ICAAMPSKVVILRYNENLSKYCIRKEIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTL
ICAAMPSKVVILRYNENLSKYCIRKEIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTL
ICAAMPSKVVILRYNENLSKYCIRKEIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTL

EEFLDKNDHSLAPAVFAASSNSFPVSIVQVNSAGQREEYLLCFHEFGVFVDSYGRRSRTD
EEFLDKNDHSLAPAVFAASSNSFPVSIVQVNSAGQREEYLLCFHEFGVFVDSYGRRSRTD
EEFLDKNDHSLAPAVFAASSNSFPVSIVQVNSAGQREEYLLCFHEFGVFVDSYGRRSRTD

DLKWSRLPLAFAYREPYLFVTHFNSLEVIEIQARSSAGTPARAYLDIPNPRYLGPAISSG
DLKWSRLPLAFAYREPYLFVTHFNSLEVIEIQARSSAGTPARAYLDIPNPRYLGPAISSG
DLKWSRLPLAFAYREPYLFVTHFNSLEVIEIQARSSAGTPARAYLDIPNPRYLGPAISSG

AIYLASSYQDKLRVICCKGNLVKESGTEHHRGPSTSRSSPNKRGPPTYNEHITKRVASSP
AIYLASSYQDKLRVICCKGNLVKESGTEHHRGPSTSRSSPNKRGPPTYNEHITKRVASSP
AIYLASSYQDKLRVICCKGNLVKESGTEHHRGPSTSRSSPNKRGPPTYNEHITKRVASSP

APPEGPSHPREPSTPHRYREGRTELRRDKSPGRPLEREKSPGRMLSTRRERSPGRLFEDS
APPEGPSHPREPSTPHRYREGRTELRRDKSPGRPLEREKSPGRMLSTRRERSPGRLFEDS
APPEGPSHPREPSTPHRYREGRTELRRDKSPGRPLEREKSPGRMLSTRRERSPGRLFEDS

SRGRLPAGAVRTPLSQVNKVWDQSSV 2054
SRGRLPAGAVRTPLSQVNKVWDQSSV
SRGRLPAGAVRTPLSQVNKVWDQSSV 1286

Fig. 10

BLASTP - alignment of 543_Protein against aageneseq|AAB43359|AAB43359
Human ORFX ORF3123 polypeptide sequence SEQ ID NO:6246.

This hit is scoring at : 0.0 (expectation value)
Alignment length (overlap) : 1286
Identities : 100 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : aageneseq

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Q:    769 VLDNQIKKDLADKETLENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSE
          VLDNQIKKDLADKETLENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSE
H:      1 VLDNQIKKDLADKETLENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSE

          ANKLAANSSLFTQRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHS
          ANKLAANSSLFTQRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHS
          ANKLAANSSLFTQRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHS

          DKNRLLELETRLREVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQ
          DKNRLLELETRLREVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQ
          DKNRLLELETRLREVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQ

          AKTELEETTAEAEEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQ
          AKTELEETTAEAEEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQ
          AKTELEETTAEAEEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQ

          NFYLSKQLDEASGANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQV
          NFYLSKQLDEASGANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQV
          NFYLSKQLDEASGANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQV

          MDLEALNDELLEKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESR
          MDLEALNDELLEKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESR
          MDLEALNDELLEKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESR

          QVVELAVKEHKAEILALQQALKEQKLKAESLSDKLNDLEKKHAMLEMNARSLQQKLETER
          QVVELAVKEHKAEILALQQALKEQKLKAESLSDKLNDLEKKHAMLEMNARSLQQKLETER
          QVVELAVKEHKAEILALQQALKEQKLKAESLSDKLNDLEKKHAMLEMNARSLQQKLETER
```

Fig. 10 (continued)

ELKQRLLEEQAKLQQQMDLQKNHIFRLTQGLQEALDRADLLKTERSDLEYQLENIQVLYS
ELKQRLLEEQAKLQQQMDLQKNHIFRLTQGLQEALDRADLLKTERSDLEYQLENIQVLYS
ELKQRLLEEQAKLQQQMDLQKNHIFRLTQGLQEALDRADLLKTERSDLEYQLENIQVLYS

HEKVKMEGTISQQTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEKEKARCAELEEALQK
HEKVKMEGTISQQTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEKEKARCAELEEALQK
HEKVKMEGTISQQTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEKEKARCAELEEALQK

TRIELRSAREEAAHRKATDHPHPSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRK
TRIELRSAREEAAHRKATDHPHPSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRK
TRIELRSAREEAAHRKATDHPHPSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRK

ESSTPEEFSRRLKERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGRQASKCLECQVMCHP
ESSTPEEFSRRLKERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGRQASKCLECQVMCHP
ESSTPEEFSRRLKERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGRQASKCLECQVMCHP

KCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQTKEPSSSLHLEGWMKVPRNNKRGQQ
KCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQTKEPSSSLHLEGWMKVPRNNKRGQQ
KCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQTKEPSSSLHLEGWMKVPRNNKRGQQ

GWDRKYIVLEGSKVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGAVGASELANTAKADV
GWDRKYIVLEGSKVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGAVGASELANTAKADV
GWDRKYIVLEGSKVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGAVGASELANTAKADV

PYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRVSREKAEADAKLLGN
PYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRVSREKAEADAKLLGN
PYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRVSREKAEADAKLLGN

SLLKLEGDDRLDMNCTLPFSDQVVLVGTEEGLYALNVLKNSLTHVPGIGAVFQIYIIKDL
SLLKLEGDDRLDMNCTLPFSDQVVLVGTEEGLYALNVLKNSLTHVPGIGAVFQIYIIKDL
SLLKLEGDDRLDMNCTLPFSDQVVLVGTEEGLYALNVLKNSLTHVPGIGAVFQIYIIKDL

EKLLMIAGEERALCLVDVKKVKQSLAQSHLPAQPDISPNIFEAVKGCHLFGAGKIENGLC
EKLLMIAGEERALCLVDVKKVKQSLAQSHLPAQPDISPNIFEAVKGCHLFGAGKIENGLC
EKLLMIAGEERALCLVDVKKVKQSLAQSHLPAQPDISPNIFEAVKGCHLFGAGKIENGLC

ICAAMPSKVVILRYNENLSKYCIRKEIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTL
ICAAMPSKVVILRYNENLSKYCIRKEIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTL
ICAAMPSKVVILRYNENLSKYCIRKEIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTL

Fig. 10 (continued)

EEFLDKNDHSLAPAVFAASSNSFPVSIVQVNSAGQREEYLLCFHEFGVFVDSYGRRSRTD
EEFLDKNDHSLAPAVFAASSNSFPVSIVQVNSAGQREEYLLCFHEFGVFVDSYGRRSRTD
EEFLDKNDHSLAPAVFAASSNSFPVSIVQVNSAGQREEYLLCFHEFGVFVDSYGRRSRTD

DLKWSRLPLAFAYREPYLFVTHFNSLEVIEIQARSSAGTPARAYLDIPNPRYLGPAISSG
DLKWSRLPLAFAYREPYLFVTHFNSLEVIEIQARSSAGTPARAYLDIPNPRYLGPAISSG
DLKWSRLPLAFAYREPYLFVTHFNSLEVIEIQARSSAGTPARAYLDIPNPRYLGPAISSG

AIYLASSYQDKLRVICCKGNLVKESGTEHHRGPSTSRSSPNKRGPPTYNEHITKRVASSP
AIYLASSYQDKLRVICCKGNLVKESGTEHHRGPSTSRSSPNKRGPPTYNEHITKRVASSP
AIYLASSYQDKLRVICCKGNLVKESGTEHHRGPSTSRSSPNKRGPPTYNEHITKRVASSP

APPEGPSHPREPSTPHRYREGRTELRRDKSPGRPLEREKSPGRMLSTRRERSPGRLFEDS
APPEGPSHPREPSTPHRYREGRTELRRDKSPGRPLEREKSPGRMLSTRRERSPGRLFEDS
APPEGPSHPREPSTPHRYREGRTELRRDKSPGRPLEREKSPGRMLSTRRERSPGRLFEDS

SRGRLPAGAVRTPLSQVNKVWDQSSV 2054
SRGRLPAGAVRTPLSQVNKVWDQSSV
SRGRLPAGAVRTPLSQVNKVWDQSSV 1286

Fig. 11

BLASTP - alignment of 543_Protein against trembl|AB023166|AB023166_1
gene: "KIAA0949"; product: "KIAA0949 protein"; Homo sapiens mRNA for KIAA0949
protein, partial cds. //:gp|AB023166|4589542 gene: "KIAA0949"; product: "KIAA0949 protein"; Homo sapiens mRNA for KIAA0949 protein, partial cds.

This hit is scoring at : 0.0 (expectation value)
Alignment length (overlap) : 940
Identities : 100 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb_1_;

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Q:   1115 QSRARADQRITESRQVVELAVKEHKAEILALQQALKEQKLKAESLSDKLNDLEKKHAMLE
          QSRARADQRITESRQVVELAVKEHKAEILALQQALKEQKLKAESLSDKLNDLEKKHAMLE
H:       1 QSRARADQRITESRQVVELAVKEHKAEILALQQALKEQKLKAESLSDKLNDLEKKHAMLE

            MNARSLQQKLETERELKQRLLEEQAKLQQQMDLQKNHIFRLTQGLQEALDRADLLKTERS
            MNARSLQQKLETERELKQRLLEEQAKLQQQMDLQKNHIFRLTQGLQEALDRADLLKTERS
            MNARSLQQKLETERELKQRLLEEQAKLQQQMDLQKNHIFRLTQGLQEALDRADLLKTERS

            DLEYQLENIQVLYSHEKVKMEGTISQQTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEK
            DLEYQLENIQVLYSHEKVKMEGTISQQTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEK
            DLEYQLENIQVLYSHEKVKMEGTISQQTKLIDFLQAKMDQPAKKKKVPLQYNELKLALEK

            EKARCAELEEALQKTRIELRSAREEAAHRKATDHPHPSTPATARQQIAMSAIVRSPEHQP
            EKARCAELEEALQKTRIELRSAREEAAHRKATDHPHPSTPATARQQIAMSAIVRSPEHQP
            EKARCAELEEALQKTRIELRSAREEAAHRKATDHPHPSTPATARQQIAMSAIVRSPEHQP

            SAMSLLAPPSSRRKESSTPEEFSRRLKERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGR
            SAMSLLAPPSSRRKESSTPEEFSRRLKERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGR
            SAMSLLAPPSSRRKESSTPEEFSRRLKERMHHNIPHRFNVGLNMRATKCAVCLDTVHFGR

            QASKCLECQVMCHPKCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQTKEPSSSLHLE
            QASKCLECQVMCHPKCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQTKEPSSSLHLE
            QASKCLECQVMCHPKCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQTKEPSSSLHLE

            GWMKVPRNNKRGQQGWDRKYIVLEGSKVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGA
            GWMKVPRNNKRGQQGWDRKYIVLEGSKVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGA
            GWMKVPRNNKRGQQGWDRKYIVLEGSKVLIYDNEAREAGQRPVEEFELCLPDGDVSIHGA
```

Fig 11 (continued)

VGASELANTAKADVPYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRV
VGASELANTAKADVPYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRV
VGASELANTAKADVPYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAGGRV

SREKAEADAKLLGNSLLKLEGDDRLDMNCTLPFSDQVVLVGTEEGLYALNVLKNSLTHVP
SREKAEADAKLLGNSLLKLEGDDRLDMNCTLPFSDQVVLVGTEEGLYALNVLKNSLTHVP
SREKAEADAKLLGNSLLKLEGDDRLDMNCTLPFSDQVVLVGTEEGLYALNVLKNSLTHVP

GIGAVFQIYIIKDLEKLLMIAGEERALCLVDVKKVKQSLAQSHLPAQPDISPNIFEAVKG
GIGAVFQIYIIKDLEKLLMIAGEERALCLVDVKKVKQSLAQSHLPAQPDISPNIFEAVKG
GIGAVFQIYIIKDLEKLLMIAGEERALCLVDVKKVKQSLAQSHLPAQPDISPNIFEAVKG

CHLFGAGKIENGLCICAAMPSKVVILRYNENLSKYCIRKEIETSEPCSCIHFTNYSILIG
CHLFGAGKIENGLCICAAMPSKVVILRYNENLSKYCIRKEIETSEPCSCIHFTNYSILIG
CHLFGAGKIENGLCICAAMPSKVVILRYNENLSKYCIRKEIETSEPCSCIHFTNYSILIG

TNKFYEIDMKQYTLEEFLDKNDHSLAPAVFAASSNSFPVSIVQVNSAGQREEYLLCFHEF
TNKFYEIDMKQYTLEEFLDKNDHSLAPAVFAASSNSFPVSIVQVNSAGQREEYLLCFHEF
TNKFYEIDMKQYTLEEFLDKNDHSLAPAVFAASSNSFPVSIVQVNSAGQREEYLLCFHEF

GVFVDSYGRRSRTDDLKWSRLPLAFAYREPYLFVTHFNSLEVIEIQARSSAGTPARAYLD
GVFVDSYGRRSRTDDLKWSRLPLAFAYREPYLFVTHFNSLEVIEIQARSSAGTPARAYLD
GVFVDSYGRRSRTDDLKWSRLPLAFAYREPYLFVTHFNSLEVIEIQARSSAGTPARAYLD

IPNPRYLGPAISSGAIYLASSYQDKLRVICCKGNLVKESGTEHHRGPSTSRSSPNKRGPP
IPNPRYLGPAISSGAIYLASSYQDKLRVICCKGNLVKESGTEHHRGPSTSRSSPNKRGPP
IPNPRYLGPAISSGAIYLASSYQDKLRVICCKGNLVKESGTEHHRGPSTSRSSPNKRGPP

TYNEHITKRVASSPAPPEGPSHPREPSTPHRYREGRTELRRDKSPGRPLEREKSPGRMLS
TYNEHITKRVASSPAPPEGPSHPREPSTPHRYREGRTELRRDKSPGRPLEREKSPGRMLS
TYNEHITKRVASSPAPPEGPSHPREPSTPHRYREGRTELRRDKSPGRPLEREKSPGRMLS

TRRERSPGRLFEDSSRGRLPAGAVRTPLSQVNKVWDQSSV 2054
TRRERSPGRLFEDSSRGRLPAGAVRTPLSQVNKVWDQSSV
TRRERSPGRLFEDSSRGRLPAGAVRTPLSQVNKVWDQSSV 940

Fig. 12

BLASTP - alignment of 543_Protein against swissnew|P54265|DMK_MOUSE MYOTONIN-PROTEIN KINASE (EC 2.7.1.-) (MYOTONIC DYSTROPHY PROTEIN KINASE) (MDPK) (DM-KINASE) (DMK) (DMPK) (MT-PK).//:swiss|P54265|DMK_MOUSE MYOTONIN-PROTE KINASE
(EC 2.7.1.-) (MYOTONIC DISTROPHY PROTEIN KINASE) (MDPK) (DM-KINASE) (DMK) (DMPK (MT-PK).//:trembl|Z38015|MMMDMPK_1 gene: "DM-PK"; product: "myotonic dystrophy protein kinase"; M.musculus DMR-N9 gene, exons 4 and 5, and DM-PK gene encoding myotonic dystrophy protein kinase //:gp|Z38015|563526 gene: "DM-PK"; product: "myotonic dystrophy protein kinase"; M.musculus DMR-N9 gene, exons 4 and 5, and DM-PK gene encoding myotonic dystrophy protein kinase.

This hit is scoring at : 3e-89 (expectation value)
Alignment length (overlap) : 522
Identities : 38 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb_1_;

```
Q:      46 LSREGILDALFVLFEECSQPALMKIKHVSNFVRKYSDTIAELQELQPSAKDFEVRSLVGC
           L. E :LD.L. :.:E.... L.: K:V::F:: .....A.L:E:: ...DFE: .::G
H:      20 LGLEPLLDLLLGVHQELGASHLAQDKYVADFLQWVEPIAARLKEVRLQRDDFEILKVIGR

           GHFAEVQVVREKATGDIYAMKVMKKKALLAQEQVSFFEEERNILSRSTSPWIPQLQYAFQ
           G F:EV.VV: K.TG.:YAMK:M.K  :L.: :VS F.EER::L :... WI.QL.:AFQ
           GAFSEVAVVKMKQTGQVYAMKIMNKWDMLKRGEVSCFREERDVLVKGDRRWITQLHFAFQ

           DKNHLYLVMEYQPGGDLLSLLNRYEDQLDENLIQFYLAELILAVHSVHLMGYVHRDIKPE
           D:N:LYLVMEY. GGDLL:LL::: :::....:.:FYLAE:::A:.SVH :GYVHRDIKP:
           DENYLYLVMEYYVGGDLLTLLSKFGERIPAEMARFYLAEIVMAIDSVHRLGYVHRDIKPD

           NILVDRTGHIKLVDFGSAAKMNSNKMVNAKLPIGTPDYMAPEVL-TVMNGDGKGTYGLDC
           NIL:DR.GHI:L.DFGS..K:..: MV.: :.:GTPDY::PE:L .V .G.G.G:YG :C
           NILLDRCGHIRLADFGSCLKLQPDGMVRSLVAVGTPDYLSPEILQAVGGGPGAGSYGPEC

           DWWSVGVIAYEMIYGRSPFAEGTSARTFNNIMNFQRFLKFP-DDPKVSSDFLDLIQSLLC
           DWW::GV.AYEM.YG::PF ..::A.T: .I:::::..L..P  D. V..:  DLI:.LLC
           DWWALGVFAYEMFYGQTPFYADSTAETYAKIVHYREHLSLPLADTVVPEEAQDLIRGLLC
```

Fig. 12 (continued)

```
GQKERLKFEG---LCCHPFFSKIDWNNIRNSPPPFVPTLKSDDDTSNFD--EPEKNSWVS
 .: RL   G   .  HPFF  :DW..:R:S PPF.P..:. .DT.NFD  E.. .:.VS
PAEIRLGRGGAGDFQKHPFFFGLDWEGLRDSVPPFTPDFEGATDTCNFDVVEDRLTAMVS

SSPCQLSPSGFS---GEELPFVGFSYSKALGILGRSESVVSGLDSPAKTSSMEKKLLIKS
..  .LS..  .   G .LPFVG:SY   . :. R...V      P .T..  :.L :.
GGGETLSDMQEDMPLGVRLPFVGYSY---CCMAFRDNQV------PDPTPMELEALQLPV

KELQDSQDKCHKMEQEMTRLHRRVSEVEAVLSQKEVELKASETQRSLLEQDLATYITECS
.:LQ. . :   ...:... .. : .V.A :::.E..:...:.Q.:L E: L..     .
SDLQGLDLQPPVSPPDQVAEEADLVAVPAPVAEAETTVTLQQLQEALEEEVLTR-----Q

SLKRSLE---QARMEVSQEDDKALQLLHDIREQSRKLQEIKE     554
SL.R.LE   .A....S.: .:A    .D:... R:LQE .E
SLSRELEAIRTANQNFSSQLQEAEVRNRDLEAHVRQLQERME     527
```

Fig. 13

BLASTP - alignment of 543_Protein against pdb|1CDK|1CDK-A
camp-dependent protein kinase(protein kinase a)protein kinase inhibitor(pki(5-24))

This hit is scoring at : 4e-44 (expectation value)
Alignment length (overlap) : 333
Identities : 33 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb_1_;

```
Q:      71 KHVSNFVRKYSDTIAELQELQPSAKDFEVRSLVGCGHFAEVQVVREKATGDIYAMKVMKK
           K  .:F::K:.:.......L.     .FE  ..:G.G.F..V.:V:.K.TG: :AMK::.K
H:      14 KAKEDFLKKWENPAQNTAHLD----QFERIKTLGTGSFGRVMLVKHKETGNHFAMKILDK

           KALLAQEQVSFFEEERNILSRSTSPWIPQLQYAFQDKNHLYLVMEYQPGGDLLSLLNRYE
           :.:::. :Q:..  .E:.IL.. . P:: :L:Y:F:D.::LY:VMEY PGG::.S L.R.
           QKVVKLKQIEHTLNEKRILQAVNFPFLVKLEYSFKDNSNLYMVMEYVPGGEMFSHLRRI-

           DQLDENLIQFYLAELILAVHSVHLMGYVHRDIKPENILVDRTGHIKLVDFGSAAKMNSNK
           .:..E  .:FY.A:::L... :H :..::RD:KPEN:L:D:.G:I::.DFG A.::....
           GRFSEPHARFYAAQIVLTFEYLHSLDLIYRDLKPENLLIDQQGYIQVTDFGFAKRVKGRT

           MVNAKLPIGTPDYMAPEVLTVMNGDGKGTYGLDCDWWSVGVIAYEMIYGRSPFAEGTSAR
           .. .    GTP:Y:APE::.      .KG Y.  .DWW::GV:.YEM. G .PF .....:
           WTLC----GTPEYLAPEIIL-----SKG-YNKAVDWWALGVLIYEMAAGYPPFFADQPIQ

           TFNNIMNFQRFLKFPDDPKVSSDFLDLIQSLLCGQKERLKFEG--------LCCHPFFSK
           .:..I:: :  ::FP.  ..SSD. DL:::LL  Q : .K  G        :  H.:F:.
           IYEKIVSGK--VRFPS--HFSSDLKDLLRNLL--QVDLTKRFGNLKDGVNDIKNHKWFAT

           IDWNNI--RNSPPPFVPTLKSDDDTSNFDEPEK     393
           .DW  I  R. ..PF:P..K...DTSNFD: E:
           TDWIAIYQRKVEAPFIPKFKGPGDTSNFDDYEE     325
```

Fig. 14

HMMPFAM - alignment of 543_Protein against pfam|hmm|pkinase
Protein kinase domain

This hit is scoring at : 219.4 E=5.5e-62
Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:      97 FEVRSLVGCGHFAEVQVVREKaTGDIYAMKVMKKKALLaqeqvsffEEERNILSRSTSPW
           :E: . :G G.F.:V. .:.K TG.I.A:K::KK::L          .E .IL.R :.P
H:       1 yelleklGeGsfGkVykakhk.tgkivAvKilkkesls........lrEiqilkrlsHpN

          IPQLQYAFQ-DKNHLYLVMEYQPGGDLLSLLNRYEdQLDENLIQFYLAELILAVHSVHLM
          I :L  .F: ..:HLYLVMEY..GGDL...L.R   .L.E. .: ...::: .:. :H
          IvrllgvfedtddhlylvmEymegGdLfdylrrng.plsekeakkialQilrGleYLHsn

          GYVHRDIKPENILVDRTGHIKLVDFGSAAKMnsnkmVNAKLPIGTPDYM-APEVLtvMNG
          G.VHRD:KPENIL:D..G :K:.DFG A. :      .... :GTP YM APEV:  :.G
          givHRDLKpeNILldengtvKiaDFGLArll.....eklttfvGTpwYmmAPEvi..leg

          dgkGTYGLDCDWWSVGVIAYEMIYG-------------------------RSPFAE---
               Y. ..D WS:GVI.YE:: G                          : PF::
          ...rgysskvDvWSlGviLyElltggplfpgadlpaftggdevdqliifvlklPfsdelp

          ----GTSARTFNNIMNfqrflKFPDDPKVSSDFLDLIQSLLC-GQKERL---KFEGLCCH
              .. ...F.  ..     :.P ....S.:. DL::..L  ...:R    . : :. H
          ktridpleelfrikkr.....rlplpsncSeelkdLlkkcLnkDPskRpGsatakeilnh

          PFF     360
          P:F
          pwf     278
```

Fig. 15

HMMPFAM - alignment of 543_Protein against pfam|hmm|PH
PH domain

This hit is scoring at : 45.8 E=1.8e-11
Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:    1471 LHLEGWMKVPRnnkrgqQGWDRKYIVLEGSKVLIYDNE-AREAGQRPVEEFELCLpDGdv
           :  EGW:  ..      :.W.::Y.VL .: :L.Y.:. .:..G. P:.  ::   ..
H:       1 vikeGwLlkks......kswkkRyfvLfnnvLlyykdskkkpkgsipLsgcqvek.pd..

           sihgavgaselantakadvPYILKMEShPHttcwpgrTLYLLAPSFPDKQRWVTALESVV
                              . ..::.: ..       TL.L A.S .::::.WV.A::S.:
           ...................kncFeirt.dr.......tlllqaeseeerkeWvkaiqsai

           A    1590
           .
           r      85
```

Fig. 16

HMMPFAM - alignment of 543_Protein against pfam|hmm|CNH
CNH domain (Domain found in NIK1-like kinase, mouse citron and yeast ROM1, ROM2)

This hit is scoring at : 380.7 1.5e-110
Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:    1619 LDMNCTLPFSDQ-----VVLVGTEEGLYALNVLKN--------SLTHVPGIGAVFQIYII
           ....C. P.: .      ::LVGTEEGLY.LN: ..         :L..: .  :V QI:::
H:       1 ytakcnhpitcdaLWGkiLLvgTeeGLYvlnisdqlnkdhfegtlekiisrrsvtqiwvl

          KDLEKLLMIAGE---ERALCLVDVKKV-----------KQSLAQSHLPAQPDISPNIFEA
          :: . LLMI:G:     A  L .: :             K.:L..:.L .:.:: .  .E
          eennvLlmisGkkpylyahpLsglvekklaqknspisikdalgsarlviRknvlsvkied

          VKG--CHLFGA-GKIEN--GLCICAAMPSKVVIL--RYNENLSKYCIR-----------
          VKG  CHLF..  . :.   L :.AA:.S.V :L   YN.  .  ..:
          vkGNSchlfavkvngkragilflaaalkssvqllaqwynplkkfklfksSNNiLNNEled

          ------KEIETSEPCSCIHFTNY---SILIGTNK----FYEIDMKQYTL----------E
                K I . . .  :  :::   .I.IG.:K       .D: Q:            .
          ikkflkklivpvpllveltsssfelpkiciGvdkPVGgeagfdvvqfhqtphlNslkfks

          EFLDKNDHSLAPAVFAASS-----NSFPVSIVQVNSAGQREEYLLCFHEFGVFVDSYG--
          ..:.K.D SL. A: ..S.      ....V IVQ :..GQR:E.LLCF.EFGVFV: .G
          slvskedlslpnaleetskkiaTCkpisviivqqsdgGqRdelLLcfdefgvfVNlqGae

          -RRSRTDDLKWSRLPLAFAYREPYLFVTHFNSLEVIEIQARSS-AGTPARA-YLDIPNPR
           RRSR.. L.W. :P AFAY EPYL.. H N.:E: EI:. .  . . A . .L:. . R
          arrsrkpiltwefmpeafayvepyllafhsngieIreietgelNlqeladrallearkir

          YLGP-AISSGAIYLASSY    1916
          .LG. .IS. .I.L:SS
          lLgsCeisdrkIllsssp     378
```

Fig. 17

HMMPFAM - alignment of 543_Protein against pfam|hmm|DAG_PE-bind
Phorbol esters/diacylglycerol binding

This hit is scoring at : 28.7 E=6.1e-05
Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:   1390 HRFNVGLN-MRATKCAVCLDTVHFG-RQASKCLECQVMCHPKCSTCLPATC   1438
          HRF.  .  ...T C  C :.:    :Q. KC .C : .H.:C.. :P..C
H:      1 HrFkrttfyksptfCdhCgellwglakQGlkCsnCglnvHkrChekVptnC     51
```

Note: Phorbol esters/diacylglycerol binding domain also as the Protein kinase C conserved region 1 (C1) domain. Diacylglycerol (DAG) is an important second messenger. Phorbol esters (PE) are analogues of DAG and potent tumor promoters that cause a variety of physiological changes when administered to both cells and tissues. DAG activates a family of serine/threonine protein kinases, collectively known as protein kinase C (PKC). Phorbol esters can directly stimulate PKC. The N-terminal region of PKC, known as C1, has been shown to bind PE and DAG in a phospholipid and zinc-dependent fashion. The C1 region contains one or two copies (depending on the isozyme of PKC) of a cysteine-rich domain about 50 amino-acid residues long and essential for DAG/PE-binding.

Fig. 18

```
HMMPFAM - alignment of 543_Protein against pfam|hmm|pkinase_C
Protein kinase C terminal domain

This hit is scoring at : 15.4   E=0.0018
Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Q:     361 SKIDWNNI--RNSPPPFVPTLKSDDDTSNFDE     390
           .:IDW:.:  :. .PPF P.:KS. DTSNFD:
H:       1 reIdWdkLEnkeiePPFKPkiksprDtsNFDk      32
```

Fig. 19

Prosite results:

PS00479	1390->1439	DAG_PE_BIND_DOM_1	PDOC00379
PS00029	854->876	LEUCINE_ZIPPER	PDOC00029
PS00029	991->1013	LEUCINE_ZIPPER	PDOC00029
PS00029	1057->1079	LEUCINE_ZIPPER	PDOC00029
PS00029	1159->1181	LEUCINE_ZIPPER	PDOC00029
PS00107	103->127	PROTEIN_KINASE_ATP	PDOC00100
PS00108	217->230	PROTEIN_KINASE_ST	PDOC00100
PS00867	1172->1180	CPSASE_2	PDOC00676

Fig. 20

genewise output:

```
gi|3599509|gb|A     1 MLKFKYGVRNPPEASASEPIASRASRLNLFFQ
                      MLKFKYG RNP +A A+EPIASRASRLNLFFQ
                      MLKFKYGARNPLDAGAAEPIASRASRLNLFFQ
gi|13653116|r1909637 atatatggcactgggggggcagacgtacacttc
                      ttataagcgactacgccactcggccgtattta
                      gggcataggttgtttttactccgccggtgccg

gi|3599509|gb|A    33                          GKPPLMTQQQMSALSREGMLDALFAL
                                               GKPP MTQQQMS LSREG+LDALF L
                                               GKPPFMTQQQMSPLSREGILDALFVL
gi|13653116|r1909733 GTAACAG  Intron 1   TAGgacctaacccatcctcggatggctgc
                      0-----[1909733:1916-0>gaccttcaaatcctcgagttactttt
                                               gaactgtagggtttcaagaatccttc

gi|3599509|gb|A    59 FEECSQPALMKMKHVSSFVQK                          SD
                      FEECSQPALMK+KHVS+FV+K                          SD
                      FEECSQPALMKIKHVSNFVRK          Y:Y[tat]        SD
gi|13653116|r1916682 tggtaccgcaaaacgaatgcaTGTAAGTT  Intron 2   CAGATtg
                      taaggaccttataatgattga 1-----[1916746:1928-1>  ca
                      taactgttgggtgcgcctcgg                          cc

gi|3599509|gb|A    83 TIAELRELQPSARDFEVRSLVGCGHFAEVQVVREKATGDVYAMKIMKKK
                      TIAEL+ELQPSA+DFEVRSLVGCGHFAEVQVVREKATGD+YAMK+MKKK
                      TIAELQELQPSAKDFEVRSLVGCGHFAEVQVVREKATGDIYAMKVMKKK
gi|13653116|r1928115 aaggtcgccctgagtggaacggtgctgggcggagagaggatgaagaaaa
                      ctcataatacccaatatggttgggatcatattgaaccgatactattaaa
                      catgaggcgtgagccacattatttcttagggaagaacgccttgaggggg

gi|3599509|gb|A   132 ALLAQEQ                          VSFFEEERNILSRSTSPWI
                      ALLAQEQ                          VSFFEEERNILSRSTSPWI
                      ALLAQEQ                          VSFFEEERNILSRSTSPWI
```

Fig. 20 (continued)

```
gi|13653116|r1928262 gttgcgcGTAGGAG  Intron 3   TAGgtttgggcaattcaaacta
                     cttcaaa0-----[1928283:1935-0>tcttaaagattcggcgcgt
                     tagcggg                        tattgaggcaatacacggc


gi|3599509|gb|A  158 PQLQYAFQDKNNLYL                          VMEYQPGGDFL
                     PQLQYAFQDKN+LYL                          VMEYQPGGD+L
                     PQLQYAFQDKNHLYL                          VMEYQPGGDLL
gi|13653116|r1935587 cctctgtcgaacctcGTGAGTC  Intron 4   CAGgagtccgggtc
                     cataactaaaaatat0-----[1935632:1951-0>ttaaacggatt
                     caagtctgcatcttg                          cgatgtagcgg


gi|3599509|gb|A  184 SLLNRYEDQLDESMIQFYLAELILAVHSVHQMGYVH
                     SLLNRYEDQLDE++IQFYLAELILAVHSVH MGYVH
                     SLLNRYEDQLDENLIQFYLAELILAVHSVHLMGYVH
gi|13653116|r1951610 tctaatggctggacacttcggcatggcagccagtgc
                     cttagaaaataaattatatcatttctagtattgata
                     atgtatgcgatacgagtcatggtgttccttggacgt


gi|3599509|gb|A  220                          DIKPENILIDRTGEIKLVDFGSA
                                              DIKPENIL+DRTG IKLVDFGSA
                                 R:R[cga]     DIKPENILVDRTGHIKLVDFGSA
gi|13653116|r1951718 CGGTAAGTG  Intron 5   CAGAgaacgaacggcagcaacggtgtg
                       2-----[1951720:1952-2> atacaatttagcgatattatgcc
                                              ccgtgctctccaaccgggttatc


gi|3599509|gb|A  244 AKMNSNKV                            -DAKLPIGTPDYMAPEVL
                     AKMNSNK+                              AKLPIGTPDYMAPEVL
                     AKMNSNKM                           VNAKLPIGTPDYMAPEVL
gi|13653116|r1953011 gaaataaaGTAAAAA  Intron 6   TAGgagaccagacgtagcggc
                     catacaat0-----[1953035:1960-0>tacatctgccaatccatt
                     gagtacgg                          gtcacgtgcatcgttagg
```

Fig. 20 (continued)

```
gi|3599509|gb|A  269 TVMNEDRRGTYGLDCDWWSVGVVAYEMVYGKTPFTEGTSARTFNNIMNF
                     TVMN D +GTYGLDCDWWSVGV+AYEM+YG++PF EGTSARTFNNIMNF
                     TVMNGDGKGTYGLDCDWWSVGVIAYEMIYGRSPFAEGTSARTFNNIMNF
gi|13653116|r1960491 agaagggagatgcgtgtttgggagtgaatgatctgggatgaataaaaat
                     cttagagagcagtagaggctgttcaattaggcctcagcccgctaattat
                     tggcgtaaccccgctcggagcgtctggttgacccagactcacctctgtc

gi|3599509|gb|A  318 Q                       RFLKFPDDPKVSSELLDLLQSLLCV
                     Q                       RFLKFPDDPKVSS+ LDL+QSLLC
                     Q                       RFLKFPDDPKVSSDFLDLIQSLLCG
gi|13653116|r1960638 cGTAAAGA  Intron 7   CAGcttatcggcagaagtcgcacatttg
                     a0-----[1960641:1962-0>gttatcaacatggattattagttgg
                     g                       gtgatatccagctctttgtacggcc

gi|3599509|gb|A  344 QKERLKFEGLCCHPFFARTDWNNIRN
                     QKERLKFEGLCCHPFF++ DWNNIRN
                     QKERLKFEGLCCHPFFSKIDWNNIRN          S:S[tct]
gi|13653116|r1962909 cagacatggcttcctttaagtaaacaTGTAAGTA  Intron 8
                     aaagtatagtggacttcatagaatga 1-----[1962988:19824
                     gagaggtattccttcctatcgccttc

gi|3599509|gb|A  370      PPPFVPTLKSDDDTSNFDEPEKNSWAFILCVPAEPLAFSGEELP
                          PPPFVPTLKSDDDTSNFDEPEKNSW         P  FSGEELP
                          PPPFVPTLKSDDDTSNFDEPEKNSWVSSSPCQLSPSGFSGEELP
gi|13653116|r1982415 CAGCTccctgcacatgggatatggcgaattgtttctccactgttgggcc
                     -1>  cccttcctacaaaccataacaaacgtccccgatgccgtcgaatc
                          tccctcccgtctccctttaaggtggtactgcggccaccgtaagg

gi|3599509|gb|A  415 FVGFSYSKALGYLGRS                          SVVSSLD
                     FVGFSYSKALG LGRS                          SVVS LD
                     FVGFSYSKALGILGRS          E:E[gag]        SVVSGLD
gi|13653116|r1982552 tggtttaagcgacgatGAGTAAGTG  Intron 9   TAGGtggtgcg
                     ttgtcagactgttggc  2-----[1982602:2000-2> cttcgta
                     tggtgccgaggtttat                         ttggtgc
```

Fig. 20 (continued)

```
gi|3599509|gb|A  439 SPAKVSSMEKKLLIKSKELQDSQDKCHK
                     SPAK SSMEKKLLIKSKELQDSQDKCHK
                     SPAKTSSMEKKLLIKSKELQDSQDKCHK
gi|13653116|r2000764 tcgaaatagaaccaaaagccgtcgatcaGTATTTA  Intron 10
                     cccacgctaaatttagaataacaaagaa0-----[2000848:20017
                     ctcgtccgagatccacagaactgcgtcg

gi|3599509|gb|A  467    MEQEMTRLHRRVSEVEAVLSQKEVELKASETQRSLLEQDLATYITE
                        MEQEMTRLHRRVSEVEAVLSQKEVELKASETQRSLLEQDLATYITE
                        MEQEMTRLHRRVSEVEAVLSQKEVELKASETQRSLLEQDLATYITE
gi|13653116|r2001753 CAGagcgaactccagtgggggcacagggcagtgacatccgcgcgataag
                     -0>taaatcgtaggtcatacttgaaatataccacagcttaaatccatca
                        gggagcgatgagagggtgttgggggggctgtgaccgggcttcccaa

gi|3599509|gb|A  513 CS                          SLKRSLEQARMEVSQEDDKALQLL
                     CS                          SLKRSLEQARMEVSQEDDKALQLL
                     CS                          SLKRSLEQARMEVSQEDDKALQLL
gi|13653116|r2001894 taGTGAGCC  Intron 11  CAGatacatgcgcaggtcgggagcccc
                     gg0-----[2001900:2003-0>gtaggtaacgtatcaaaaactatt
                     ct                          cagatggaaggggcggtcaaggtc

gi|3599509|gb|A  539 HDIREQSRKLQEIKEQ                              EYQAQVEEMR
                     HDIREQSRKLQEIKEQ                              EYQAQVEEMR
                     HDIREQSRKLQEIKEQ                              EYQAQVEEMR
gi|13653116|r2003242 cgaagcacaccgaagcGTAGGCC  Intron 12  TAGgtcgcgggaa
                     aatgaaggataataaa0-----[2003290:2008-0>aaacataatg
                     ttcaggcggcaacagg                              gcgtagaagg

gi|3599509|gb|A  565 LMMNQLEEDLVSARRRSDLYESELRESRLAAEEFKRKANECQHKLMK
                     LMMNQLEEDLVSARRRSDLYESELRESRLAAEEFKRKA ECQHKL+K
                     LMMNQLEEDLVSARRRSDLYESELRESRLAAEEFKRKATECQHKLLK
gi|13653116|r2008995 taaactgggcgtgaacagctgtgcagtccggggtacagagtccacta
                     tttaataaattccggggataacatgacgtccaatagaccagaaatta
                     gggtggagttcaaaagttccatggagtgtttaacggagaatgtaggg
```

Fig. 20 (continued)

```
gi|3599509|gb|A  612                              AKDQGKPEVGEYSKLEK
                                                  AKDQGKPEVGEY+KLEK
                                                  AKDQGKPEVGEYAKLEK
gi|13653116|r2009136 GTAGTCA  Intron 13  CAGgagcgacggggtgacga
                     0-----[2009136:2009-0>caaagacatgaacataa
                                                  tgtaggtagaatgaggg


gi|3599509|gb|A  629                              INAEQQLKIQELQEKLEK
                                                  INAEQQLKIQELQEKLEK
                                                  INAEQQLKIQELQEKLEK
gi|13653116|r2009450 GTATACT  Intron 14  TAGaaggcccaacgccgacga
                     0-----[2009450:2009-0>tacaaatataataaataa
                                                  cttgggcatggcagaggg


gi|3599509|gb|A  647                              AVKASTEATELLQNIRQAKERAEREL
                                                  AVKASTEATELLQNIRQAKERAEREL
                                                  AVKASTEATELLQNIRQAKERAEREL
gi|13653116|r2010022 GTAAGCC  Intron 15  TAGggagaaggagcccaaccgagcggagc
                     0-----[2010022:2012-0>ctacgcaccattaatgacaagcagat
                                                  taaccggccggggtccgaggacgggg


gi|3599509|gb|A  673 EKLHNREDSSEGIKKKLVEAE                          ERRHS
                     EKL NREDSSEGI+KKLVEAE                          ERRHS
                     EKLQNREDSSEGIRKKLVEAE                          ERRHS
gi|13653116|r2012975 gaccacggttggaaaacggggGTGAGCA  Intron 16  CAGgccct
                     aataagaaccagtgaattaca0-----[2013038:2014-0>aggac
                     ggggcagtttaccaggggatg                          acctt


gi|3599509|gb|A  699 LENKVKRLETMERRENRLKDDIQTKSEQIQQMADKIL
                     LENKVKRLETMERRENRLKDDIQTKS+QIQQMADKIL
                     LENKVKRLETMERRENRLKDDIQTKSQQIQQMADKIL
gi|13653116|r2014912 cgaagaacgaagcagaacaggacaatccaccaggaac
                     taaatagtactaggaagtaaatacacaataatcaatt
                     ggcgagaagcggtaacaggtccgaacagcgggttatg
```

Fig. 20 (continued)

```
gi|3599509|gb|A  736                              ELEEKHREAQVSAQHLEVHLKQKEQH
                                                  ELEEKHREAQVSAQHLEVHLKQKEQH
                                                  ELEEKHREAQVSAQHLEVHLKQKEQH
gi|13653116|r2015023 GTGAGCA  Intron 17  TAGgcggaccggcgtgcccggccacagcc
                     0-----[2015023:2018-0>ataaaagacatccaatatataaaaaa
                                                  gcagatggcacacgcaagcgagaggc

gi|3599509|gb|A  762 YEEKIK                          VLDNQIKKDLADKESLENMM
                     YEEKIK                          VLDNQIKKDLADKE+LENMM
                     YEEKIK                          VLDNQIKKDLADKETLENMM
gi|13653116|r2018703 tggaaaGTAAAGA  Intron 18  TAGgtgacaaagcggagacgaaa
                     aaaata0-----[2018721:2024-0>ttaaataaatcaaactaatt
                     tgagta                          ggctgagacgtcggaggcgg

gi|3599509|gb|A  788 QRHEEEAHEKGKILSEQKA                          MINAMDS
                     QRHEEEAHEKGKILSEQKA                          MINAMDS
                     QRHEEEAHEKGKILSEQKA                          MINAMDS
gi|13653116|r2024812 cacggggcgagaacagcagGTAGGTA  Intron 19  CAGaaagagt
                     agaaaacaaagattgaaac0-----[2024869:2027-0>ttactac
                     gacgggctggcatccaggg                          gcttgtc

gi|3599509|gb|A  814 KIRSLEQRIVELSEANKLAANSSLFTQRN
                     KIRSLEQRIVELSEANKLAANSSLFTQRN
                     KIRSLEQRIVELSEANKLAANSSLFTQRN
gi|13653116|r2027128 aaatcgcaaggctggaacggaaactacaa
                     atgctaagttatcacaatccaggttcaga
                     gcacgaggtgagtactataatctttcagc

gi|3599509|gb|A  843                                KAQEEMISELRQQKFYLETQAGK
                                                    KAQEEMISELRQQKFYLETQAGK
                                 M:M[atg]           KAQEEMISELRQQKFYLETQAGK
gi|13653116|r2027215 ATGTAAGTA  Intron 20  CAGGagcggaatgcaccattcgacgga
                       2-----[2027217:2028-2> acaaattcatgaaatatacacga
                                                    gcaaggttacgagatcggagtgg
```

Fig. 20 (continued)

```
gi|3599509|gb|A  867 LEAQNRKLEEQLEKISHQDHSDKSRLLELETRLRE
                     LEAQNRKLEEQLEKISHQDHSDK+RLLELETRLRE
                     LEAQNRKLEEQLEKISHQDHSDKNRLLELETRLRE
gi|13653116|r2028332 tggcacacggccgaaaccgcagaacccgcgaatcg
                     tacaagataaataatgaaaagaaagttatacgtga
                     ggcgcaaggggggcccacctcgtgggaggaaggg

gi|3599509|gb|A  902                           VSLEHEEQKLELKRQLTELQLSLQER
                                               VSLEHEEQKLELKRQLTELQLSLQER
                                               VSLEHEEQKLELKRQLTELQLSLQER
gi|13653116|r2028437 GTGAGAG  Intron 21  CAGgacgcggcacgcacccagccctccgc
                     0-----[2028437:2033-0>tgtaaaaaatatagatcatatctaag
                                               ctagcgggaggcgcgcagagccgggc

gi|3599509|gb|A  928 ESQLTALQAARAALESQLRQAKTELEETTAEAEEEIQALT
                     ESQLTALQAARAALESQLRQAKTELEETTAEAEEEIQALT
                     ESQLTALQAARAALESQLRQAKTELEETTAEAEEEIQALT
gi|13653116|r2033637 gtctagccggcggcgaccccgaagcggaaggggggacgca
                     acatcctaccgcctagatgacacataacccacaaatactc
                     gaggacggtaggcggcgtcgggaggagcaaataggcgacg

gi|3599509|gb|A  968                             AHRDEIQRKFDALRNSCT
                                                 AHRDEIQRKFDALRNSCT
                                                 AHRDEIQRKFDALRNSCT
gi|13653116|r2033757 GTAGGTC  Intron 22  TAGgcaggaccatggccaata
                     0-----[2033757:2043-0>cagaatagatactgaggc
                                                 atatacgcatttttccttt

gi|3599509|gb|A  986                             VITDLEEQLNQLTEDNAELNNQNFYL
                                                 VITDLEEQLNQLTEDNAELNNQNFYL
                                                 VITDLEEQLNQLTEDNAELNNQNFYL
gi|13653116|r2043396 GTGAGTA  Intron 23  TAGgaagcggccaccaggaggcaacattt
                     0-----[2043396:2050-0>ttcataaataatcaaacataaaatat
                                                 acacggggacggcgcctacccacccg
```

Fig. 20 (continued)

```
gi|3599509|gb|A 1012 SKQLDEASGANDEIVQLRSEVDHLRREITEREMQLTSQKQ
                     SKQLDEASGANDEIVQLRSEVDHLRREITEREMQLTSQKQ
                     SKQLDEASGANDEIVQLRSEVDHLRREITEREMQLTSQKQ
gi|13653116|r2050527 taccgggtggaggagcccagggccccgaagcgaccaacac
                     caataaccgcaaattatggataatggatcagatatcgaaa
                     caactgttccccgtaagatagctccggcgaagggtccgga


gi|3599509|gb|A 1052                          TMEALKTTCTMLEEQVLDLEALNDEL
                                              TMEALKTTCTMLEEQV+DLEALNDEL
                                              TMEALKTTCTMLEEQVMDLEALNDEL
gi|13653116|r2050647 GTAAGGA  Intron 24  CAGaaggcaaataacggcgagtggcaggc
                     0-----[2050647:2051-0>ctactaccgcttaaattatactaaat
                                              gggtggcgccgggagcgtggcactgg


gi|3599509|gb|A 1078 LEKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQS
                     LEKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQS
                     LEKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQS
gi|13653116|r2051527 cgagcctggtaagcgggatctgtcgcgccaacgagaca
                     taaagagacgggttgaaacataggtgatagttacaaag
                     aaaggggcggccgttgacgtgtgtagggaggccgagc


gi|3599509|gb|A 1116                               ARADQRITESRQVVELAVKEHKA
                                                   ARADQRITESRQVVELAVKEHKA
                                 R:R[agg]          ARADQRITESRQVVELAVKEHKA
gi|13653116|r2051641 AGGTGGGGC  Intron 25  CAGGgaggccaagtccgggcggagcag
                       2-----[2051643:2055-2> cgcaagtcacgattatctaaaac
                                                   gactggccgtcgggggagggcgt


gi|3599509|gb|A 1140 EILALQQALKEQKLKAESLSDK                          LNDL
                     EILALQQALKEQKLKAESLSDK                          LNDL
                     EILALQQALKEQKLKAESLSDK                          LNDL
gi|13653116|r2055246 gacgcccgcagcacaggactgaGTCAGCG  Intron 26  TAGcagc
                     attctaactaaaatacagtcaa0-----[2055312:2057-0>taat
                     gtctgggtcagggggcgcctcg                          ctcg
```

Fig. 20 (continued)

```
gi|3599509|gb|A 1166 EKKHAMLEMNARSLQQKLETERELKQRLLEE
                     EKKHAMLEMNARSLQQKLETERELKQRLLEE
                     EKKHAMLEMNARSLQQKLETERELKQRLLEE
gi|13653116|r2057212 gaacgacgaagcatccacgagcgcacaccgg
                     aaaacttatacggtaaatacagataagttaa
                     gggttgtagtcacaggggggtaagcaggtgag

gi|3599509|gb|A 1197                              QAKLQQQMDLQKNHIFRLTQGLQEAL
                                                  QAKLQQQMDLQKNHIFRLTQGLQEAL
                                                  QAKLQQQMDLQKNHIFRLTQGLQEAL
gi|13653116|r2057305 GTGAGTG  Intron 27  TAGcgatcccagccaacatccacgccggc
                     0-----[2057305:2064-0>acataaatataaaattgtcagtaact
                                                  acaaggggcggatctctgtaagaata

gi|3599509|gb|A 1223 DRADLLKTERSDLEYQLENIQ                          VLYSH
                     DRADLLKTERSDLEYQLENIQ                          VLYSH
                     DRADLLKTERSDLEYQLENIQ                          VLYSH
gi|13653116|r2064435 gcggccaagaagtgtccgaacGTGAGGA  Intron 28  TAGgcttc
                     agcattacaggataaataata0-----[2064498:2065-0>ttaca
                     tgttaggaaatcggtggactg                          tcttt

gi|3599509|gb|A 1249 EKVKMEGTISQQTKLIDFLQAKMDQPAKKKK
                     EKVKMEGTISQQTKLIDFLQAKMDQPAKKKK
                     EKVKMEGTISQQTKLIDFLQAKMDQPAKKKK
gi|13653116|r2065236 gagaaggaatccaacagtccgaagccgaaaa
                     aatatagctcaacattattacataaccaaaa
                     aggagactttaacactttgacagcattagag

gi|3599509|gb|A 1280                              VPLQYNELKLALEKEKARCAELEEAL
                                                  VPLQYNELKLALEKEKARCAELEEAL
                                                  VPLQYNELKLALEKEKARCAELEEAL
gi|13653116|r2065329 GTGAGTC  Intron 29  CAGgccctagcacgcgagagctggcgggc
                     0-----[2065329:2066-0>tctaaaatatctaaaacggcataact
                                                  ttggctggggcggggatctagagact
```

Fig. 20 (continued)

```
gi|3599509|gb|A 1306 QKTRIELRSAREE                              AHRKATDHPH
                     QKTRIELRSAREE                              AHRKATDHPH
                     QKTRIELRSAREE          A:A[gct]            AHRKATDHPH
gi|13653116|r2067068 caacagcctgcggGGTAGGGG  Intron 30  CAGCTgccagagccc
                     aacgtatgccgaa 1-----[2067108:2067-1>  cagaccaaca
                     ggcccgcgccgga                              cccaagccac

gi|3599509|gb|A 1330 PSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRKESSTPE
                     PSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRKESSTPE
                     PSTPATARQQIAMSAIVRSPEHQPSAMSLLAPPSSRRKESSTPE
gi|13653116|r2067429 ctacgagaccagatgagctcgcccagaaccgcctacaagttacg
                     ccccccgaatctccttgccaaacgctgttccccgggaacccca
                     acgaccggggccgcccgggagcgctcgcggcgacccaggtatag

gi|3599509|gb|A 1374                            FSRRLKERMHHNIPHRFNVGLNM
                                                FSRRLKERMHHNIPHRFNVGLNM
                               E:E[gaa]         FSRRLKERMHHNIPHRFNVGLNM
gi|13653116|r2067561 GGTACGTT  Intron 31  CAGAAtacccagcaccaaccctaggcaa
                      1-----[2067562:2071-1>  tgggtaagtaaatcagtatgtat
                                              ttgttgacgcctttcaccaagcg

gi|3599509|gb|A 1398 RATKCAVCLDTVHFGRQASKCL                          C
                     RATKCAVCLDTVHFGRQASKCL                          C
                     RATKCAVCLDTVHFGRQASKCL          E:E[gaa]        C
gi|13653116|r2071543 cgaatggtcgagctgccgtatcGGTAAGAT  Intron 32  TAGAAt
                     gccagctgtactatggaccagt 1-----[2071610:2072-1>  g
                     acagttgtgtcgctacgacatc                          t

gi|3599509|gb|A 1422 QVMCHPKCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQSKEPGSSL
                     QVMCHPKCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQ+KEP SSL
                     QVMCHPKCSTCLPATCGLPAEYATHFTEAFCRDKMNSPGLQTKEPSSSL
gi|13653116|r2072163 cgatccattattcgatgtcggtgactaggttcgaaatcgccaagcaaat
                     attgacagccgtcccggtccaaccatcactggaataccgtacaacgggt
                     gggtccgccgcgaccccgttatcacccgccctcagccatcgcggccccg
```

Fig. 20 (continued)

```
gi|3599509|gb|A 1471 HLEGWMKVP                              NNKRGQQGWDRKYI
                     HLEGWMKVP                              NNKRGQQGWDRKYI
                     HLEGWMKVP            R:R[agg]          NNKRGQQGWDRKYI
gi|13653116|r2072310 ccggtaagcAGGTACCAT  Intron 33  CAGGaaacgccgtgaata
                     ataggtatc  2-----[2072339:2072-2> aaaggaaggagaat
                     cgaggggggc                             tcaaagacgcggct

gi|3599509|gb|A 1495 VLEGSKVLIYDNEARE                         GQRPVEE
                     VLEGSKVLIYDNEARE                         GQRPVEE
                     VLEGSKVLIYDNEARE          A:A[gct]       GQRPVEE
gi|13653116|r2072555 gcggtagcatgaggagGGTAAATT  Intron 34  AAGCTgcacggg
                     ttagcatttaaaacga 1-----[2072604:2073-1>  gagctaa
                     cggaaaccttctacaa                         aggggaa

gi|3599509|gb|A 1519 FELCLPDGDVSIHGAVGASELANTAKA
                     FELCLPDGDVSIHGAVGASELANTAKA
                     FELCLPDGDVSIHGAVGASELANTAKA          D:D[gat]
gi|13653116|r2073134 tgctccggggtacgggggtgcgaagagGGTGAGGA  Intron 35
                     tatgtcagatctagctgccatcaccac 1-----[2073216:20734
                     tggctccgtattttctttcacatacaa

gi|3599509|gb|A 1546      VPYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAG
                          VPYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAG
                          VPYILKMESHPHTTCWPGRTLYLLAPSFPDKQRWVTALESVVAG
gi|13653116|r2073456 TAGATgctacaagtcccaattcgaacttcgcatcgacctgagtgtgggg
                     -1>  tcattatacacaccggcggctattccgtcaaaggtcctacttcg
                          cacagggatcgccccgcgacccgatccctcagcgcccaaatcat

gi|3599509|gb|A 1591 GRVSREKAEADA                            KLLGNSLLKLEGDD
                     GRVSREKAEADA                            KLLGNSLLKLEGDD
                     GRVSREKAEADA                            KLLGNSLLKLEGDD
gi|13653116|r2073593 gagtagaggggGTGAGTA  Intron 36  AAGaccgatccacgggg
                     ggtcgaacacac0-----[2073629:2075-0>attgacttatagaa
                     gattgaaaattt                            agtaccggagattc
```

Fig. 20 (continued)

```
gi|3599509|gb|A 1617 RLDMNCTLPFSDQ                          VVLVGTEEGLYAL
                     RLDMNCTLPFSDQ                          VVLVGTEEGLYAL
                     RLDMNCTLPFSDQ                          VVLVGTEEGLYAL
gi|13653116|r2075228 ccgaatacctagcGTAATGC  Intron 37  CAGggtggagggctgc
                     gtatagctctgaa0-----[2075267:2075-0>ttttgcaagtact
                     tacgccggcctcg                          ggggccgagcccg


gi|3599509|gb|A 1643 NVLKNSLTHIPGIGAVFQIYIIKDLEKLLMIA
                     NVLKNSLTH+PGIGAVFQIYIIKDLEKLLMIA
                     NVLKNSLTHVPGIGAVFQIYIIKDLEKLLMIA
gi|13653116|r2075459 agtaatcacgcgagggtcataaagcgaccaag
                     attaactcatcgtgcttatattaataattttc
                     tcgaccactcaataaccatttcgcgggacgaa


gi|3599509|gb|A 1675                          EERALCLVDVKKVKQSLAQSHLP
                                              EERALCLVDVKKVKQSLAQSHLP
                              G:G[gga]        EERALCLVDVKKVKQSLAQSHLP
gi|13653116|r2075555 GGTGTGAG  Intron 38  CAGGAggcgctcgggaagactcgctccc
                      1-----[2075556:2077-1>  aagctgttataataactcacatc
                                              aggagttgcggagagcgcgccgt


gi|3599509|gb|A 1699 AQPDVSPNIFEAVKGCHLFAAGK                    IEN
                     AQPD+SPNIFEAVKGCHLF AGK                    IEN
                     AQPDISPNIFEAVKGCHLFGAGK                    IEN
gi|13653116|r2077559 gccgatcaatgggagtcttgggaGTAAGCT  Intron 39  CAGaga
                     cacatccattactaggattgcga0-----[2077628:2081-0>taa
                     cgcccaccttatcgcccgtgacg                    tgc


gi|3599509|gb|A 1725 SLCICAAMPSKVVILRYNDNLSKYCIRK
                      LCICAAMPSKVVILRYN+NLSKYCIRK
                     GLCICAAMPSKVVILRYNENLSKYCIRK
gi|13653116|r2081360 gctatggacaaggacctagacaattacaGTAAGTC  Intron 40
                     gtgtgcctcgattttgaaaatgaagtga0-----[2081444:20838
                     gccctacgccacctccccacccacccga
```

Fig. 20 (continued)

```
gi|3599509|gb|A 1753     EIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTLD
                         EIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTL+
                         EIETSEPCSCIHFTNYSILIGTNKFYEIDMKQYTLE
gi|13653116|r2083845 CAGgagatgctatactaataacagaaattgagaactacg
                     -0>ataccacgggtatcaagtttgcaataatataaacta
                        gagcagccctcccctctcctactaccaccgggcgcg

gi|3599509|gb|A 1789                              FLDKNDHSLAPAVFASSSNSFPV
                                                  FLDKNDHSLAPAVFA+SSNSFPV
                                 E:E[gaa]         FLDKNDHSLAPAVFAASSNSFPV
gi|13653116|r2083956 GGTAGGAC  Intron 41  CAGAAtcgaagcttgcggtggttaatcg
                      1-----[2083957:2084-1>  ttaaaaactcccttccccagtct
                                              cgtgtctcgattgtcctcccctc

gi|3599509|gb|A 1813 SIVQANSAGQREEYLLCFH                          FGVF
                     SIVQ NSAGQREEYLLCFH                          FGVF
                     SIVQVNSAGQREEYLLCFH          E:E[gaa]        FGVF
gi|13653116|r2084125 tagcgaaggccggttcttcGGTGAGTC  Intron 42  CAGAAtggt
                     cttatagcgagaaattgta 1-----[2084183:2084-1>  tgtt
                     acgggccaggaggcggtcc                         tagc

gi|3599509|gb|A 1837 VDSYGRRSRTDDLKWSRLPLAF                        Y
                     VDSYGRRSRTDDLKWSRLPLAF                        Y
                     VDSYGRRSRTDDLKWSRLPLAF          A:A[gcc]      Y
gi|13653116|r2084995 ggttgacacaggcatactctgtGGTACGTG  Intron 43  CAGCCt
                     tacaggggcaatagggtctct 1-----[2085062:2087-1>  a
                     gttcaatccactcggtcatgct                        c

gi|3599509|gb|A 1861 REPYLFVTHFNSLEVIEIQARSSL
                     REPYLFVTHFNSLEVIEIQARSS
                     REPYLFVTHFNSLEVIEIQARSSA          G:G[ggg]
gi|13653116|r2087757 agctctgactatcggagacgcttgGGGTAAGCA  Intron 44
                     gacatttcatactattatacgccc  2-----[2087831:20879
                     aactgtgccccacaatgcgaccaa
```

Fig. 20 (continued)

```
gi|3599509|gb|A 1885      SPARAYLEIPNPRYLGPAISSGAIYLASSYQDKLRVICCKGNLVK
                          +PARAYL+IPNPRYLGPAISSGAIYLASSYQDKLRVICCKGNLVK
                          TPARAYLDIPNPRYLGPAISSGAIYLASSYQDKLRVICCKGNLVK
gi|13653116|r2087965 CAGGacgcgtcgacacctcgcgattggattgtttcgatagattagacga
                     -2> cccgcatatcacgatgcctccgctatcccaaaatgttggagatta
                         ctcagcgccgcgccgctctcaagtcggcacgtaagctccgaccgg


gi|3599509|gb|A 1931 ESGTEQHRVPSTSR                              SPNKRGPPT
                     ESGTE HR PSTSR                               SPNKRGPPT
                     ESGTEHHRGPSTSR           S:S[agc]            SPNKRGPPT
gi|13653116|r2088104 gtgagcccgctatcAGGTAACCA  Intron 45  CAGCacaacgcca
                     acgcaaaggccccg  2-----[2088148:2095-2> gcaaggccc
                     gcctaccgcgcccc                               cccgacacg


gi|3599509|gb|A 1955 YNEHITKRVASSPAPPEGPSHPREPSTPHRYRDREGRTELRRDKSPGRP
                     YNEHITKRVASSPAPPEGPSHPREPSTPHRYR  EGRTELRRDKSPGRP
                     YNEHITKRVASSPAPPEGPSHPREPSTPHRYR--EGRTELRRDKSPGRP
gi|13653116|r2095380 tagcaaacggtacgccggcacccgcaaccctc  ggcagccagatcgcc
                     aaaatcagtccgccccagcgacgacgccagag  aggcatggaaccggc
                     ccgcccgcgcccaggcaccccgagacaccccc  gggcggcgcgttccc


gi|3599509|gb|A 2004 LEREKSPGRMLSTRRERSPGRLFEDSSRGRLPAGAVRTPLSQVNKVWDQ
                     LEREKSPGRMLSTRRERSPGRLFEDSSRGRLPAGAVRTPLSQVNKV
                     LEREKSPGRMLSTRRERSPGRLFEDSSRGRLPAGAVRTPLSQVNKVRQH
gi|13653116|r2095521 cgcgatcgcacaacagctcgactggaaagcccggggaacctcgaagacc
                     tagaaccggttgcggagccggttaaggggtccgctgcctcataatgaa
                     ggaggcccggccggaggccgggtacccgcggtgacggcggcggcggggt


gi|3599509|gb|A 2053 S
                     S
                     S
gi|13653116|r2095668 t
                     c
                     c
```

Fig. 20 (continued)

```
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          match    1909637 2095670
3906.49
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      1909637 1909732
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   1909733 1916603
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      1916604 1916745
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   1916746 1928106
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      1928107 1928282
0.00
+       2       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   1928283 1935529
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      1935530 1935631
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   1935632 1951576
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      1951577 1951719
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   1951720 1952940
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      1952941 1953034
0.00
+       1       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   1953035 1960436
0.00
+       .       gi|3599509|gb|AAC72823.1|
```

Fig. 20 (continued)

gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 1960437 1960640
0.00
+ 0 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 1960641 1962833
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 1962834 1962987
0.00
+ 0 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 1962988 1982417
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 1982418 1982601
0.00
+ 2 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 1982602 2000741
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2000742 2000847
0.00
+ 1 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2000848 2001755
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2001756 2001899
0.00
+ 0 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2001900 2003169
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2003170 2003289
0.00
+ 0 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2003290 2008964
0.00
+ . gi|3599509|gb|AAC72823.1|

Fig. 20 (continued)

gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2008965 2009135
0.00
+ . 0 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2009136 2009398
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2009399 2009449
0.00
+ 0 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2009450 2009967
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2009968 2010021
0.00
+ 0 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2010022 2012896
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2012897 2013037
0.00
+ 0 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2013038 2014896
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2014897 2015022
0.00
+ 0 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2015023 2018624
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2018625 2018720
0.00
+ 0 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2018721 2024751
0.00
+ . gi|3599509|gb|AAC72823.1|

Fig. 20 (continued)

```
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2024752 2024868
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2024869 2027106
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2027107 2027216
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2027217 2028261
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2028262 2028436
0.00
+       1       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2028437 2033558
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2033559 2033756
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2033757 2043341
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2043342 2043395
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2043396 2050448
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2050449 2050646
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2050647 2051448
0.00
+       .       gi|3599509|gb|AAC72823.1|
```

Fig. 20 (continued)

```
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2051449 2051642
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2051643 2055175
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2055176 2055311
0.00
+       1       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2055312 2057199
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2057200 2057304
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2057305 2064356
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2064357 2064497
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2064498 2065220
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2065221 2065328
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2065329 2066989
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2066990 2067107
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2067108 2067396
0.00
+       .       gi|3599509|gb|AAC72823.1|
```

Fig. 20 (continued)

gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2067397 2067561
0.00
+ 2 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2067562 2071471
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2071472 2071609
0.00
+ 2 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2071610 2072157
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2072158 2072338
0.00
+ 2 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2072339 2072511
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2072512 2072603
0.00
+ 1 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2072604 2073110
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2073111 2073215
0.00
+ 2 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2073216 2073458
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2073459 2073628
0.00
+ 2 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2073629 2075185
0.00
+ . gi|3599509|gb|AAC72823.1|

Fig. 20 (continued)

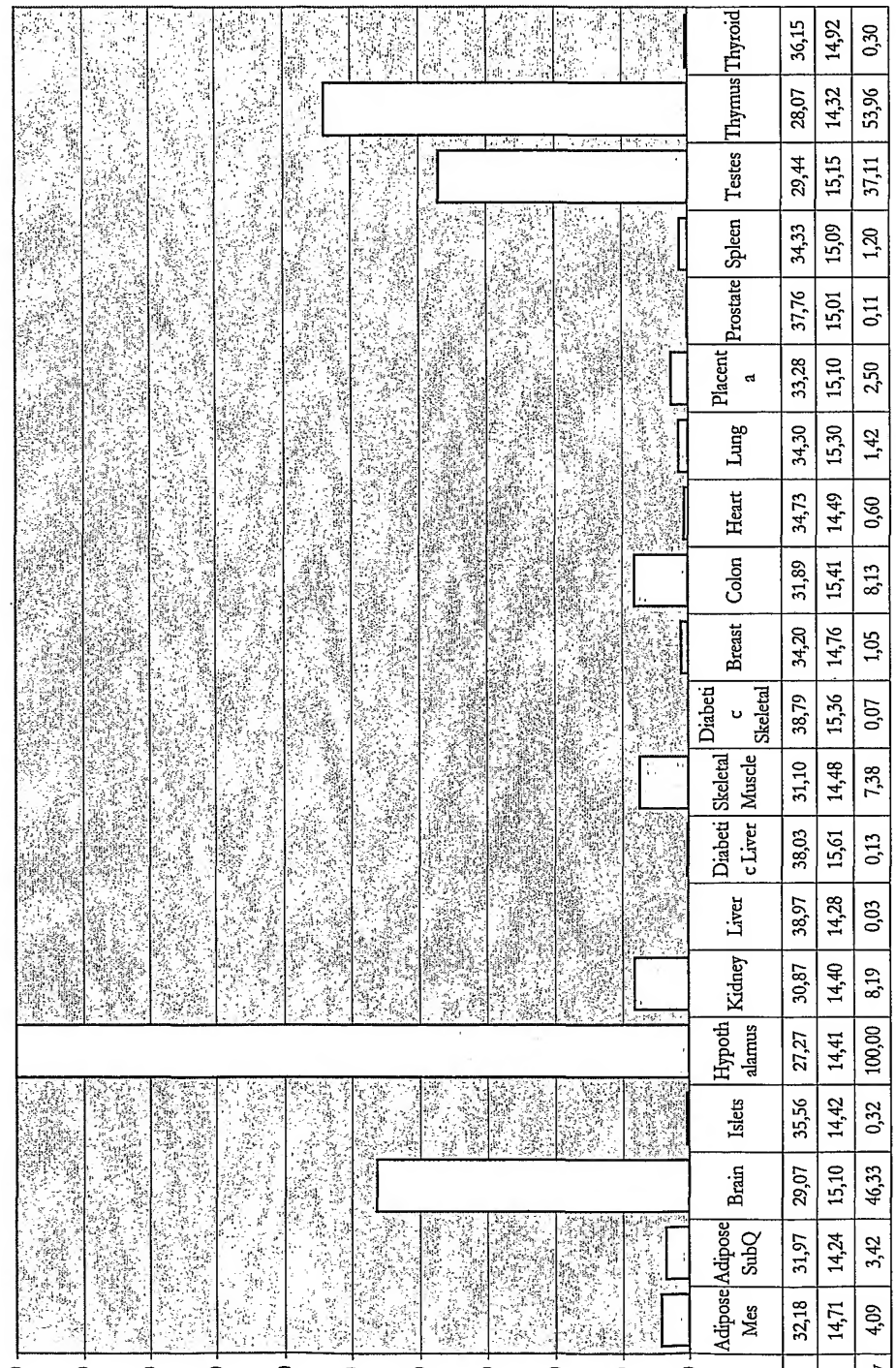
```
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2075186 2075266
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2075267 2075419
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2075420 2075555
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2075556 2077487
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2077488 2077627
0.00
+       2       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2077628 2081350
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2081351 2081443
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2081444 2083847
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2083848 2083956
0.00
+       0       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2083957 2084053
0.00
+       .       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          cds      2084054 2084182
0.00
+       2       gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932    GeneWise          intron   2084183 2084980
0.00
+       .       gi|3599509|gb|AAC72823.1|
```

Fig. 20 (continued)

gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2084981 2085061
0.00
+ 2 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2085062 2087751
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2087752 2087830
0.00
+ 2 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2087831 2087967
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2087968 2088147
0.00
+ 1 gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise intron 2088148 2095351
0.00
+ . gi|3599509|gb|AAC72823.1|
gi|13653116|ref|NT_009775.3|Hs12_9932 GeneWise cds 2095352 2095670
0.00
+ 1 gi|3599509|gb|AAC72823.1|

FIG. 21

LBRI 543: Relative Expression



	Adipose Mes	Adipose SubQ	Brain	Islets	Hypothalamus	Kidney	Liver	Diabetic Liver	Skeletal Muscle	Diabetic Skeletal	Breast	Colon	Heart	Lung	Placenta	Prostate	Spleen	Testes	Thymus	Thyroid
543-Ct	32,18	31,97	29,07	35,56	27,27	30,87	38,97	38,03	31,10	38,79	34,20	31,89	34,73	34,30	33,28	37,76	34,33	29,44	28,07	36,15
18s-Ct	14,71	14,24	15,10	14,42	14,41	14,40	14,28	15,61	14,48	15,36	14,76	15,41	14,49	15,30	15,10	15,01	15,09	15,15	14,32	14,92
Relative Intensity	4,09	3,42	46,33	0,32	100,00	8,19	0,03	0,13	7,38	0,07	1,05	8,13	0,60	1,42	2,50	0,11	1,20	37,11	53,96	0,30

Fig. 22

TBLASTN - alignment of 543_Protein against emnew|AX166510|AX166510
Sequence 1 from Patent WO0138503.//:gbnew|AX166510|AX166510 Sequence 1 fro
Patent WO0138503.

This hit is scoring at : 0.0 (expectation value)
Alignment length (overlap) : 2053
Identities : 99 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Hit reading frame : +1
Database searched : nrnee_1_;

```
Q:      1 MLKFKYGARNPLDAGAAEPIASRASRLNLFFQGKPPFMTQQQMSPLSREGILDALFVLFE
          MLKFKYGARNPLDAGAAEPIASRASRLNLFFQGKPPFMTQQQMSPLSREGILDALFVLFE
H:      1 MLKFKYGARNPLDAGAAEPIASRASRLNLFFQGKPPFMTQQQMSPLSREGILDALFVLFE

            ECSQPALMKIKHVSNFVRK-YSDTIAELQELQPSAKDFEVRSLVGCGHFAEVQVVREKAT
            ECSQPALMKIKHVSNFV : YSDTIAELQELQPSAKDFEVRSLVGCGHFAEVQVVREKAT
            ECSQPALMKIKHVSNFVPEVYSDTIAELQELQPSAKDFEVRSLVGCGHFAEVQVVREKAT

            GDIYAMKVMKKKALLAQEQVSFFEEERNILSRSTSPWIPQLQYAFQDKNHLYLVMEYQPG
            GDIYAMKVMKKKALLAQEQVSFFEEERNILSRSTSPWIPQLQYAFQDKNHLYLVMEYQPG
            GDIYAMKVMKKKALLAQEQVSFFEEERNILSRSTSPWIPQLQYAFQDKNHLYLVMEYQPG

            GDLLSLLNRYEDQLDENLIQFYLAELILAVHSVHLMGYVHRDIKPENILVDRTGHIKLVD
            GDLLSLLNRYEDQLDENLIQFYLAELILAVHSVHLMGYVHRDIKPENILVDRTGHIKLVD
            GDLLSLLNRYEDQLDENLIQFYLAELILAVHSVHLMGYVHRDIKPENILVDRTGHIKLVD

            FGSAAKMNSNKMVNAKLPIGTPDYMAPEVLTVMNGDGKGTYGLDCDWWSVGVIAYEMIYG
            FGSAAKMNSNKMVNAKLPIGTPDYMAPEVLTVMNGDGKGTYGLDCDWWSVGVIAYEMIYG
            FGSAAKMNSNKMVNAKLPIGTPDYMAPEVLTVMNGDGKGTYGLDCDWWSVGVIAYEMIYG

            RSPFAEGTSARTFNNIMNFQRFLKFPDDPKVSSDFLDLIQSLLCGQKERLKFEGLCCHPF
            RSPFAEGTSARTFNNIMNFQRFLKFPDDPKVSSDFLDLIQSLLCGQKERLKFEGLCCHPF
            RSPFAEGTSARTFNNIMNFQRFLKFPDDPKVSSDFLDLIQSLLCGQKERLKFEGLCCHPF

            FSKIDWNNIRNSPPPFVPTLKSDDDTSNFDEPEKNSWVSSSPCQLSPSGFSGEELPFVGF
            FSKIDWNNIRNSPPPFVPTLKSDDDTSNFDEPEKNSWVSSSPCQLSPSGFSGEELPFVGF
            FSKIDWNNIRNSPPPFVPTLKSDDDTSNFDEPEKNSWVSSSPCQLSPSGFSGEELPFVGF
```

Fig. 22 (continued)

SYSKALGILGRSESVVSGLDSPAKTSSMEKKLLIKSKELQDSQDKCHKMEQEMTRLHRRV
SYSKALGILGRSESVVSGLDSPAKTSSMEKKLLIKSKELQDSQDKCHKMEQEMTRLHRRV
SYSKALGILGRSESVVSGLDSPAKTSSMEKKLLIKSKELQDSQDKCHKMEQEMTRLHRRV

SEVEAVLSQKEVELKASETQRSLLEQDLATYITECSSLKRSLEQARMEVSQEDDKALQLL
SEVEAVLSQKEVELKASETQRSLLEQDLATYITECSSLKRSLEQARMEVSQEDDKALQLL
SEVEAVLSQKEVELKASETQRSLLEQDLATYITECSSLKRSLEQARMEVSQEDDKALQLL

HDIREQSRKLQEIKEQEYQAQVEEMRLMMNQLEEDLVSARRRSDLYESELRESRLAAEEF
HDIREQSRKLQEIKEQEYQAQVEEMRLMMNQLEEDLVSARRRSDLYESELRESRLAAEEF
HDIREQSRKLQEIKEQEYQAQVEEMRLMMNQLEEDLVSARRRSDLYESELRESRLAAEEF

KRKATECQHKLLKAKDQGKPEVGEYAKLEKINAEQQLKIQELQEKLEKAVKASTEATELL
KRKATECQHKLLKAKDQGKPEVGEYAKLEKINAEQQLKIQELQEKLEKAVKASTEATELL
KRKATECQHKLLKAKDQGKPEVGEYAKLEKINAEQQLKIQELQEKLEKAVKASTEATELL

QNIRQAKERAERELEKLQNREDSSEGIRKKLVEAEERRHSLENKVKRLETMERRENRLKD
QNIRQAKERAERELEKLQNREDSSEGIRKKLVEAEERRHSLENKVKRLETMERRENRLKD
QNIRQAKERAERELEKLQNREDSSEGIRKKLVEAEERRHSLENKVKRLETMERRENRLKD

DIQTKSQQIQQMADKILELEEKHREAQVSAQHLEVHLKQKEQHYEEKIKVLDNQIKKDLA
DIQTKSQQIQQMADKILELEEKHREAQVSAQHLEVHLKQKEQHYEEKIKVLDNQIKKDLA
DIQTKSQQIQQMADKILELEEKHREAQVSAQHLEVHLKQKEQHYEEKIKVLDNQIKKDLA

DKETLENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSEANKLAANSSLF
DKETLENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSEANKLAANSSLF
DKETLENMMQRHEEEAHEKGKILSEQKAMINAMDSKIRSLEQRIVELSEANKLAANSSLF

TQRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHSDKNRLLELETR
TQRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHSDKNRLLELETR
TQRNMKAQEEMISELRQQKFYLETQAGKLEAQNRKLEEQLEKISHQDHSDKNRLLELETR

LREVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQAKTELEETTAE
LREVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQAKTELEETTAE
LREVSLEHEEQKLELKRQLTELQLSLQERESQLTALQAARAALESQLRQAKTELEETTAE

AEEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQNFYLSKQLDEA
AEEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQNFYLSKQLDEA
AEEEIQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQNFYLSKQLDEA

Fig. 22 (continued)

SGANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQVMDLEALNDELL
SGANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQVMDLEALNDELL
SGANDEIVQLRSEVDHLRREITEREMQLTSQKQTMEALKTTCTMLEEQVMDLEALNDELL

EKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESRQVVELAVKEHK
EKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESRQVVELAVKEHK
EKERQWEAWRSVLGDEKSQFECRVRELQRMLDTEKQSRARADQRITESRQVVELAVKEHK

AEILALQQALKEQKLKAESLSDKLNDLEKKHAMLEMNARSLQQKLETERELKQRLLEEQA
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Fig. 22 (continued)

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TPLSQVNKV ..S
TPLSQVNKVRQHS 6159

Fig. 23

TBLASTN - alignment of 543_Protein against BAYER_LIB_DNA|wu_373006001280181
Bayer Corp Pharma Proprietary OP Library: Fat Rat Hypothalamus Linda Wu Fr
Oct 15 15:45:51 EDT 1999

This hit is scoring at : 2e-37 (expectation value)
Alignment length (overlap) : 77
Identities : 100 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Hit reading frame : -3
Database searched : bayerall_1_;

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H:    231 IQALTAHRDEIQRKFDALRNSCTVITDLEEQLNQLTEDNAELNNQNFYLSKQLDEASGAN

          DEIVQLRSEVDHLRREI    1040
          DEIVQLRSEVDHLRREI
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Fig. 24

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Fig. 24 (continued)

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Fig. 24 (continued)

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cagcccgaca tctcacccaa catttttgaa gctgtcaagg gctgccactt gtttggggca 5160

ggcaagattg agaacgggct ctgcatctgt gcagccatgc ccagcaaagt cgtcattctc 5220

cgctacaacg aaaacctcag caaatactgc atccggaaag agatagagac ctcagagccc 5280

tgcagctgta tccacttcac caattacagt atcctcattg gaaccaataa attctacgaa 5340

atcgacatga agcagtacac gctcgaggaa ttcctggata agaatgacca ttccttggca 5400

cctgctgtgt ttgccgcctc ttccaacagc ttccctgtct caatcgtgca ggtgaacagc 5460

gcagggcagc gagaggagta cttgctgtgt ttccacgaat ttggagtgtt cgtggattct 5520

tacggaagac gtagccgcac agacgatctc aagtggagtc gcttaccttt ggcctttgcc 5580

tacagagaac cctatctgtt tgtgacccac ttcaactcac tcgaagtaat tgagatccag 5640

gcacgctcct cagcagggac ccctgcccga gcgtacctgg acatcccgaa cccgcgctac 5700

ctgggccctg ccatttcctc aggagcgatt tacttggcgt cctcatacca ggataaatta 5760

agggtcattt gctgcaaggg aaacctcgtg aaggagtccg gcactgaaca ccaccggggc 5820

ccgtccacct cccgcagcag ccccaacaag cgaggcccac ccacgtacaa cgagcacatc 5880

accaagcgcg tggcctccag cccagcgccg cccgaaggcc ccagccaccc gcgagagcca 5940

agcacacccc accgctaccg cgaggggcgg accgagctgc gcagggacaa gtctcctggc 6000

cgccccctgg agcgagagaa gtcccccggc cggatgctca gcacgcggag agagcggtcc 6060

cccgggaggc tgtttgaaga cagcagcagg ggccggctgc ctgcgggagc cgtgaggacc 6120

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<211> 2054
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<400> 2

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Met Leu Lys Phe Lys Tyr Gly Ala Arg Asn Pro Leu Asp Ala Gly Ala
1               5                  10                  15

Ala Glu Pro Ile Ala Ser Arg Ala Ser Arg Leu Asn Leu Phe Phe Gln
            20                  25                  30

Gly Lys Pro Pro Phe Met Thr Gln Gln Gln Met Ser Pro Leu Ser Arg
        35                  40                  45

Glu Gly Ile Leu Asp Ala Leu Phe Val Leu Phe Glu Glu Cys Ser Gln
    50                  55                  60

Pro Ala Leu Met Lys Ile Lys His Val Ser Asn Phe Val Arg Lys Tyr
65                  70                  75                  80

Ser Asp Thr Ile Ala Glu Leu Gln Glu Leu Gln Pro Ser Ala Lys Asp
                85                  90                  95

Phe Glu Val Arg Ser Leu Val Gly Cys Gly His Phe Ala Glu Val Gln
            100                 105                 110

Val Val Arg Glu Lys Ala Thr Gly Asp Ile Tyr Ala Met Lys Val Met
        115                 120                 125

Lys Lys Lys Ala Leu Leu Ala Gln Glu Gln Val Ser Phe Phe Glu Glu
    130                 135                 140

Glu Arg Asn Ile Leu Ser Arg Ser Thr Ser Pro Trp Ile Pro Gln Leu
145                 150                 155                 160
```

```
Gln Tyr Ala Phe Gln Asp Lys Asn His Leu Tyr Leu Val Met Glu Tyr
                165                 170                 175

Gln Pro Gly Gly Asp Leu Leu Ser Leu Leu Asn Arg Tyr Glu Asp Gln
            180                 185                 190

Leu Asp Glu Asn Leu Ile Gln Phe Tyr Leu Ala Glu Leu Ile Leu Ala
        195                 200                 205

Val His Ser Val His Leu Met Gly Tyr Val His Arg Asp Ile Lys Pro
    210                 215                 220

Glu Asn Ile Leu Val Asp Arg Thr Gly His Ile Lys Leu Val Asp Phe
225                 230                 235                 240

Gly Ser Ala Ala Lys Met Asn Ser Asn Lys Met Val Asn Ala Lys Leu
                245                 250                 255

Pro Ile Gly Thr Pro Asp Tyr Met Ala Pro Glu Val Leu Thr Val Met
            260                 265                 270

Asn Gly Asp Gly Lys Gly Thr Tyr Gly Leu Asp Cys Asp Trp Trp Ser
        275                 280                 285

Val Gly Val Ile Ala Tyr Glu Met Ile Tyr Gly Arg Ser Pro Phe Ala
    290                 295                 300

Glu Gly Thr Ser Ala Arg Thr Phe Asn Asn Ile Met Asn Phe Gln Arg
305                 310                 315                 320
```

```
Phe Leu Lys Phe Pro Asp Asp Pro Lys Val Ser Ser Asp Phe Leu Asp
                325                 330                 335

Leu Ile Gln Ser Leu Leu Cys Gly Gln Lys Glu Arg Leu Lys Phe Glu
            340                 345                 350

Gly Leu Cys Cys His Pro Phe Phe Ser Lys Ile Asp Trp Asn Asn Ile
        355                 360                 365

Arg Asn Ser Pro Pro Pro Phe Val Pro Thr Leu Lys Ser Asp Asp Asp
    370                 375                 380

Thr Ser Asn Phe Asp Glu Pro Glu Lys Asn Ser Trp Val Ser Ser Ser
385                 390                 395                 400

Pro Cys Gln Leu Ser Pro Ser Gly Phe Ser Gly Glu Glu Leu Pro Phe
                405                 410                 415

Val Gly Phe Ser Tyr Ser Lys Ala Leu Gly Ile Leu Gly Arg Ser Glu
            420                 425                 430

Ser Val Val Ser Gly Leu Asp Ser Pro Ala Lys Thr Ser Ser Met Glu
        435                 440                 445

Lys Lys Leu Leu Ile Lys Ser Lys Glu Leu Gln Asp Ser Gln Asp Lys
    450                 455                 460

Cys His Lys Met Glu Gln Glu Met Thr Arg Leu His Arg Arg Val Ser
465                 470                 475                 480
```

```
Glu Val Glu Ala Val Leu Ser Gln Lys Glu Val Glu Leu Lys Ala Ser
                485                 490                 495

Glu Thr Gln Arg Ser Leu Leu Glu Gln Asp Leu Ala Thr Tyr Ile Thr
            500                 505                 510

Glu Cys Ser Ser Leu Lys Arg Ser Leu Glu Gln Ala Arg Met Glu Val
        515                 520                 525

Ser Gln Glu Asp Asp Lys Ala Leu Gln Leu Leu His Asp Ile Arg Glu
    530                 535                 540

Gln Ser Arg Lys Leu Gln Glu Ile Lys Glu Gln Glu Tyr Gln Ala Gln
545                 550                 555                 560

Val Glu Glu Met Arg Leu Met Met Asn Gln Leu Glu Glu Asp Leu Val
                565                 570                 575

Ser Ala Arg Arg Arg Ser Asp Leu Tyr Glu Ser Glu Leu Arg Glu Ser
            580                 585                 590

Arg Leu Ala Ala Glu Glu Phe Lys Arg Lys Ala Thr Glu Cys Gln His
        595                 600                 605

Lys Leu Leu Lys Ala Lys Asp Gln Gly Lys Pro Glu Val Gly Glu Tyr
    610                 615                 620

Ala Lys Leu Glu Lys Ile Asn Ala Glu Gln Gln Leu Lys Ile Gln Glu
625                 630                 635                 640
```

```
Leu Gln Glu Lys Leu Glu Lys Ala Val Lys Ala Ser Thr Glu Ala Thr
                645                 650                 655

Glu Leu Leu Gln Asn Ile Arg Gln Ala Lys Glu Arg Ala Glu Arg Glu
            660                 665                 670

Leu Glu Lys Leu Gln Asn Arg Glu Asp Ser Ser Glu Gly Ile Arg Lys
        675                 680                 685

Lys Leu Val Glu Ala Glu Glu Arg Arg His Ser Leu Glu Asn Lys Val
    690                 695                 700

Lys Arg Leu Glu Thr Met Glu Arg Arg Glu Asn Arg Leu Lys Asp Asp
705                 710                 715                 720

Ile Gln Thr Lys Ser Gln Gln Ile Gln Gln Met Ala Asp Lys Ile Leu
                725                 730                 735

Glu Leu Glu Glu Lys His Arg Glu Ala Gln Val Ser Ala Gln His Leu
            740                 745                 750

Glu Val His Leu Lys Gln Lys Glu Gln His Tyr Glu Glu Lys Ile Lys
        755                 760                 765

Val Leu Asp Asn Gln Ile Lys Lys Asp Leu Ala Asp Lys Glu Thr Leu
    770                 775                 780

Glu Asn Met Met Gln Arg His Glu Glu Glu Ala His Glu Lys Gly Lys
785                 790                 795                 800
```

```
Ile Leu Ser Glu Gln Lys Ala Met Ile Asn Ala Met Asp Ser Lys Ile
                805                 810                 815

Arg Ser Leu Glu Gln Arg Ile Val Glu Leu Ser Glu Ala Asn Lys Leu
            820                 825                 830

Ala Ala Asn Ser Ser Leu Phe Thr Gln Arg Asn Met Lys Ala Gln Glu
        835                 840                 845

Glu Met Ile Ser Glu Leu Arg Gln Gln Lys Phe Tyr Leu Glu Thr Gln
    850                 855                 860

Ala Gly Lys Leu Glu Ala Gln Asn Arg Lys Leu Glu Glu Gln Leu Glu
865                 870                 875                 880

Lys Ile Ser His Gln Asp His Ser Asp Lys Asn Arg Leu Leu Glu Leu
                885                 890                 895

Glu Thr Arg Leu Arg Glu Val Ser Leu Glu His Glu Glu Gln Lys Leu
            900                 905                 910

Glu Leu Lys Arg Gln Leu Thr Glu Leu Gln Leu Ser Leu Gln Glu Arg
        915                 920                 925

Glu Ser Gln Leu Thr Ala Leu Gln Ala Ala Arg Ala Ala Leu Glu Ser
    930                 935                 940

Gln Leu Arg Gln Ala Lys Thr Glu Leu Glu Glu Thr Thr Ala Glu Ala
945                 950                 955                 960
```

```
Glu Glu Glu Ile Gln Ala Leu Thr Ala His Arg Asp Glu Ile Gln Arg
                965                 970                 975

Lys Phe Asp Ala Leu Arg Asn Ser Cys Thr Val Ile Thr Asp Leu Glu
            980                 985                 990

Glu Gln Leu Asn Gln Leu Thr Glu  Asp Asn Ala Glu Leu  Asn Asn Gln
        995                 1000                1005

Asn Phe  Tyr Leu Ser Lys Gln  Leu Asp Glu Ala Ser  Gly Ala Asn
    1010                1015                1020

Asp Glu  Ile Val Gln Leu Arg  Ser Glu Val Asp His  Leu Arg Arg
    1025                1030                1035

Glu Ile  Thr Glu Arg Glu Met  Gln Leu Thr Ser Gln  Lys Gln Thr
    1040                1045                1050

Met Glu  Ala Leu Lys Thr Thr  Cys Thr Met Leu Glu  Glu Gln Val
    1055                1060                1065

Met Asp  Leu Glu Ala Leu Asn  Asp Glu Leu Leu Glu  Lys Glu Arg
    1070                1075                1080

Gln Trp  Glu Ala Trp Arg Ser  Val Leu Gly Asp Glu  Lys Ser Gln
    1085                1090                1095

Phe Glu  Cys Arg Val Arg Glu  Leu Gln Arg Met Leu  Asp Thr Glu
    1100                1105                1110
```

```
Lys Gln  Ser Arg Ala Arg Ala  Asp Gln Arg Ile Thr  Glu Ser Arg
    1115                 1120                 1125

Gln Val  Val Glu Leu Ala Val  Lys Glu His Lys Ala  Glu Ile Leu
    1130                 1135                 1140

Ala Leu  Gln Gln Ala Leu Lys  Glu Gln Lys Leu Lys  Ala Glu Ser
    1145                 1150                 1155

Leu Ser  Asp Lys Leu Asn Asp  Leu Glu Lys Lys His  Ala Met Leu
    1160                 1165                 1170

Glu Met  Asn Ala Arg Ser Leu  Gln Gln Lys Leu Glu  Thr Glu Arg
    1175                 1180                 1185

Glu Leu  Lys Gln Arg Leu Leu  Glu Glu Gln Ala Lys  Leu Gln Gln
    1190                 1195                 1200

Gln Met  Asp Leu Gln Lys Asn  His Ile Phe Arg Leu  Thr Gln Gly
    1205                 1210                 1215

Leu Gln  Glu Ala Leu Asp Arg  Ala Asp Leu Leu Lys  Thr Glu Arg
    1220                 1225                 1230

Ser Asp  Leu Glu Tyr Gln Leu  Glu Asn Ile Gln Val  Leu Tyr Ser
    1235                 1240                 1245

His Glu  Lys Val Lys Met Glu  Gly Thr Ile Ser Gln  Gln Thr Lys
    1250                 1255                 1260
```

```
Leu Ile  Asp Phe Leu Gln Ala  Lys Met Asp Gln Pro  Ala Lys Lys
    1265                 1270                 1275

Lys Lys  Val Pro Leu Gln Tyr  Asn Glu Leu Lys Leu  Ala Leu Glu
    1280                 1285                 1290

Lys Glu  Lys Ala Arg Cys Ala  Glu Leu Glu Glu Ala  Leu Gln Lys
    1295                 1300                 1305

Thr Arg  Ile Glu Leu Arg Ser  Ala Arg Glu Glu Ala  Ala His Arg
    1310                 1315                 1320

Lys Ala  Thr Asp His Pro His  Pro Ser Thr Pro Ala  Thr Ala Arg
    1325                 1330                 1335

Gln Gln  Ile Ala Met Ser Ala  Ile Val Arg Ser Pro  Glu His Gln
    1340                 1345                 1350

Pro Ser  Ala Met Ser Leu Leu  Ala Pro Pro Ser Ser  Arg Arg Lys
    1355                 1360                 1365

Glu Ser  Ser Thr Pro Glu Glu  Phe Ser Arg Arg Leu  Lys Glu Arg
    1370                 1375                 1380

Met His  His Asn Ile Pro His  Arg Phe Asn Val Gly  Leu Asn Met
    1385                 1390                 1395

Arg Ala  Thr Lys Cys Ala Val  Cys Leu Asp Thr Val  His Phe Gly
    1400                 1405                 1410
```

```
Arg Gln  Ala Ser Lys Cys Leu  Glu Cys Gln Val Met  Cys His Pro
    1415                 1420                 1425

Lys Cys  Ser Thr Cys Leu Pro  Ala Thr Cys Gly Leu  Pro Ala Glu
    1430                 1435                 1440

Tyr Ala  Thr His Phe Thr Glu  Ala Phe Cys Arg Asp  Lys Met Asn
    1445                 1450                 1455

Ser Pro  Gly Leu Gln Thr Lys  Glu Pro Ser Ser Ser  Leu His Leu
    1460                 1465                 1470

Glu Gly  Trp Met Lys Val Pro  Arg Asn Asn Lys Arg  Gly Gln Gln
    1475                 1480                 1485

Gly Trp  Asp Arg Lys Tyr Ile  Val Leu Glu Gly Ser  Lys Val Leu
    1490                 1495                 1500

Ile Tyr  Asp Asn Glu Ala Arg  Glu Ala Gly Gln Arg  Pro Val Glu
    1505                 1510                 1515

Glu Phe  Glu Leu Cys Leu Pro  Asp Gly Asp Val Ser  Ile His Gly
    1520                 1525                 1530

Ala Val  Gly Ala Ser Glu Leu  Ala Asn Thr Ala Lys  Ala Asp Val
    1535                 1540                 1545

Pro Tyr  Ile Leu Lys Met Glu  Ser His Pro His Thr  Thr Cys Trp
    1550                 1555                 1560
```

```
Pro Gly  Arg Thr Leu Tyr Leu  Leu Ala Pro Ser Phe  Pro Asp Lys
    1565                 1570                 1575

Gln Arg  Trp Val Thr Ala Leu  Glu Ser Val Val Ala  Gly Gly Arg
    1580                 1585                 1590

Val Ser  Arg Glu Lys Ala Glu  Ala Asp Ala Lys Leu  Leu Gly Asn
    1595                 1600                 1605

Ser Leu  Leu Lys Leu Glu Gly  Asp Asp Arg Leu Asp  Met Asn Cys
    1610                 1615                 1620

Thr Leu  Pro Phe Ser Asp Gln  Val Val Leu Val Gly  Thr Glu Glu
    1625                 1630                 1635

Gly Leu  Tyr Ala Leu Asn Val  Leu Lys Asn Ser Leu  Thr His Val
    1640                 1645                 1650

Pro Gly  Ile Gly Ala Val Phe  Gln Ile Tyr Ile Ile  Lys Asp Leu
    1655                 1660                 1665

Glu Lys  Leu Leu Met Ile Ala  Gly Glu Glu Arg Ala  Leu Cys Leu
    1670                 1675                 1680

Val Asp  Val Lys Lys Val Lys  Gln Ser Leu Ala Gln  Ser His Leu
    1685                 1690                 1695

Pro Ala  Gln Pro Asp Ile Ser  Pro Asn Ile Phe Glu  Ala Val Lys
    1700                 1705                 1710
```

```
Gly Cys  His Leu Phe Gly Ala  Gly Lys Ile Glu Asn  Gly Leu Cys
    1715                 1720                 1725

Ile Cys  Ala Ala Met Pro Ser  Lys Val Val Ile Leu  Arg Tyr Asn
    1730                 1735                 1740

Glu Asn  Leu Ser Lys Tyr Cys  Ile Arg Lys Glu Ile  Glu Thr Ser
    1745                 1750                 1755

Glu Pro  Cys Ser Cys Ile His  Phe Thr Asn Tyr Ser  Ile Leu Ile
    1760                 1765                 1770

Gly Thr  Asn Lys Phe Tyr Glu  Ile Asp Met Lys Gln  Tyr Thr Leu
    1775                 1780                 1785

Glu Glu  Phe Leu Asp Lys Asn  Asp His Ser Leu Ala  Pro Ala Val
    1790                 1795                 1800

Phe Ala  Ala Ser Ser Asn Ser  Phe Pro Val Ser Ile  Val Gln Val
    1805                 1810                 1815

Asn Ser  Ala Gly Gln Arg Glu  Glu Tyr Leu Leu Cys  Phe His Glu
    1820                 1825                 1830

Phe Gly  Val Phe Val Asp Ser  Tyr Gly Arg Arg Ser  Arg Thr Asp
    1835                 1840                 1845

Asp Leu  Lys Trp Ser Arg Leu  Pro Leu Ala Phe Ala  Tyr Arg Glu
    1850                 1855                 1860
```

```
Pro Tyr  Leu Phe Val Thr His  Phe Asn Ser Leu Glu  Val Ile Glu
    1865                 1870                 1875

Ile Gln  Ala Arg Ser Ser Ala  Gly Thr Pro Ala Arg  Ala Tyr Leu
    1880                 1885                 1890

Asp Ile  Pro Asn Pro Arg Tyr  Leu Gly Pro Ala Ile  Ser Ser Gly
    1895                 1900                 1905

Ala Ile  Tyr Leu Ala Ser Ser  Tyr Gln Asp Lys Leu  Arg Val Ile
    1910                 1915                 1920

Cys Cys  Lys Gly Asn Leu Val  Lys Glu Ser Gly Thr  Glu His His
    1925                 1930                 1935

Arg Gly  Pro Ser Thr Ser Arg  Ser Ser Pro Asn Lys  Arg Gly Pro
    1940                 1945                 1950

Pro Thr  Tyr Asn Glu His Ile  Thr Lys Arg Val Ala  Ser Ser Pro
    1955                 1960                 1965

Ala Pro  Pro Glu Gly Pro Ser  His Pro Arg Glu Pro  Ser Thr Pro
    1970                 1975                 1980

His Arg  Tyr Arg Glu Gly Arg  Thr Glu Leu Arg Arg  Asp Lys Ser
    1985                 1990                 1995

Pro Gly  Arg Pro Leu Glu Arg  Glu Lys Ser Pro Gly  Arg Met Leu
    2000                 2005                 2010
```

Ser Thr Arg Arg Glu Arg Ser Pro Gly Arg Leu Phe Glu Asp Ser
2015 2020 2025

Ser Arg Gly Arg Leu Pro Ala Gly Ala Val Arg Thr Pro Leu Ser
2030 2035 2040

Gln Val Asn Lys Val Trp Asp Gln Ser Ser Val
2045 2050

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Met Leu Lys Phe Lys Tyr Gly Val Arg Asn Pro Pro Glu Ala Ser Ala
1 5 10 15

Ser Glu Pro Ile Ala Ser Arg Ala Ser Arg Leu Asn Leu Phe Phe Gln
20 25 30

Gly Lys Pro Pro Leu Met Thr Gln Gln Gln Met Ser Ala Leu Ser Arg
35 40 45

Glu Gly Met Leu Asp Ala Leu Phe Ala Leu Phe Glu Glu Cys Ser Gln
50 55 60

Pro Ala Leu Met Lys Met Lys His Val Ser Ser Phe Val Gln Lys Tyr
65 70 75 80

```
Ser Asp Thr Ile Ala Glu Leu Arg Glu Leu Gln Pro Ser Ala Arg Asp
                 85                  90                  95

Phe Glu Val Arg Ser Leu Val Gly Cys Gly His Phe Ala Glu Val Gln
            100                 105                 110

Val Val Arg Glu Lys Ala Thr Gly Asp Val Tyr Ala Met Lys Ile Met
        115                 120                 125

Lys Lys Lys Ala Leu Leu Ala Gln Glu Gln Val Ser Phe Phe Glu Glu
    130                 135                 140

Glu Arg Asn Ile Leu Ser Arg Ser Thr Ser Pro Trp Ile Pro Gln Leu
145                 150                 155                 160

Gln Tyr Ala Phe Gln Asp Lys Asn Asn Leu Tyr Leu Val Met Glu Tyr
                165                 170                 175

Gln Pro Gly Gly Asp Phe Leu Ser Leu Leu Asn Arg Tyr Glu Asp Gln
            180                 185                 190

Leu Asp Glu Ser Met Ile Gln Phe Tyr Leu Ala Glu Leu Ile Leu Ala
        195                 200                 205

Val His Ser Val His Gln Met Gly Tyr Val His Arg Asp Ile Lys Pro
    210                 215                 220

Glu Asn Ile Leu Ile Asp Arg Thr Gly Glu Ile Lys Leu Val Asp Phe
225                 230                 235                 240
```

```
Gly Ser Ala Ala Lys Met Asn Ser Asn Lys Val Asp Ala Lys Leu Pro
                245                 250                 255

Ile Gly Thr Pro Asp Tyr Met Ala Pro Glu Val Leu Thr Val Met Asn
            260                 265                 270

Glu Asp Arg Arg Gly Thr Tyr Gly Leu Asp Cys Asp Trp Trp Ser Val
        275                 280                 285

Gly Val Val Ala Tyr Glu Met Val Tyr Gly Lys Thr Pro Phe Thr Glu
    290                 295                 300

Gly Thr Ser Ala Arg Thr Phe Asn Asn Ile Met Asn Phe Gln Arg Phe
305                 310                 315                 320

Leu Lys Phe Pro Asp Asp Pro Lys Val Ser Ser Glu Leu Leu Asp Leu
                325                 330                 335

Leu Gln Ser Leu Leu Cys Val Gln Lys Glu Arg Leu Lys Phe Glu Gly
            340                 345                 350

Leu Cys Cys His Pro Phe Phe Ala Arg Thr Asp Trp Asn Asn Ile Arg
        355                 360                 365

Asn Ser Pro Pro Pro Phe Val Pro Thr Leu Lys Ser Asp Asp Asp Thr
    370                 375                 380

Ser Asn Phe Asp Glu Pro Glu Lys Asn Ser Trp Ala Phe Ile Leu Cys
385                 390                 395                 400
```

```
Val Pro Ala Glu Pro Leu Ala Phe Ser Gly Glu Glu Leu Pro Phe Val
                405                 410                 415

Gly Phe Ser Tyr Ser Lys Ala Leu Gly Tyr Leu Gly Arg Ser Glu Ser
            420                 425                 430

Val Val Ser Ser Leu Asp Ser Pro Ala Lys Val Ser Ser Met Glu Lys
        435                 440                 445

Lys Leu Leu Ile Lys Ser Lys Glu Leu Gln Asp Ser Gln Asp Lys Cys
    450                 455                 460

His Lys Met Glu Gln Glu Met Thr Arg Leu His Arg Arg Val Ser Glu
465                 470                 475                 480

Val Glu Ala Val Leu Ser Gln Lys Glu Val Glu Leu Lys Ala Ser Glu
                485                 490                 495

Thr Gln Arg Ser Leu Leu Glu Gln Asp Leu Ala Thr Tyr Ile Thr Glu
            500                 505                 510

Cys Ser Ser Leu Lys Arg Ser Leu Glu Gln Ala Arg Met Glu Val Ser
        515                 520                 525

Gln Glu Asp Asp Lys Ala Leu Gln Leu Leu His Asp Ile Arg Glu Gln
    530                 535                 540

Ser Arg Lys Leu Gln Glu Ile Lys Glu Gln Glu Tyr Gln Ala Gln Val
545                 550                 555                 560
```

```
Glu Glu Met Arg Leu Met Met Asn Gln Leu Glu Glu Asp Leu Val Ser
                565                 570                 575

Ala Arg Arg Arg Ser Asp Leu Tyr Glu Ser Glu Leu Arg Glu Ser Arg
            580                 585                 590

Leu Ala Ala Glu Glu Phe Lys Arg Lys Ala Asn Glu Cys Gln His Lys
        595                 600                 605

Leu Met Lys Ala Lys Asp Gln Gly Lys Pro Glu Val Gly Glu Tyr Ser
    610                 615                 620

Lys Leu Glu Lys Ile Asn Ala Glu Gln Gln Leu Lys Ile Gln Glu Leu
625                 630                 635                 640

Gln Glu Lys Leu Glu Lys Ala Val Lys Ala Ser Thr Glu Ala Thr Glu
                645                 650                 655

Leu Leu Gln Asn Ile Arg Gln Ala Lys Glu Arg Ala Glu Arg Glu Leu
            660                 665                 670

Glu Lys Leu His Asn Arg Glu Asp Ser Ser Glu Gly Ile Lys Lys Lys
        675                 680                 685

Leu Val Glu Ala Glu Glu Arg Arg His Ser Leu Glu Asn Lys Val Lys
    690                 695                 700

Arg Leu Glu Thr Met Glu Arg Arg Glu Asn Arg Leu Lys Asp Asp Ile
705                 710                 715                 720
```

```
Gln Thr Lys Ser Glu Gln Ile Gln Gln Met Ala Asp Lys Ile Leu Glu
                725                 730                 735

Leu Glu Glu Lys His Arg Glu Ala Gln Val Ser Ala Gln His Leu Glu
            740                 745                 750

Val His Leu Lys Gln Lys Glu Gln His Tyr Glu Glu Lys Ile Lys Val
        755                 760                 765

Leu Asp Asn Gln Ile Lys Lys Asp Leu Ala Asp Lys Glu Ser Leu Glu
    770                 775                 780

Asn Met Met Gln Arg His Glu Glu Glu Ala His Glu Lys Gly Lys Ile
785                 790                 795                 800

Leu Ser Glu Gln Lys Ala Met Ile Asn Ala Met Asp Ser Lys Ile Arg
                805                 810                 815

Ser Leu Glu Gln Arg Ile Val Glu Leu Ser Glu Ala Asn Lys Leu Ala
            820                 825                 830

Ala Asn Ser Ser Leu Phe Thr Gln Arg Asn Met Lys Ala Gln Glu Glu
        835                 840                 845

Met Ile Ser Glu Leu Arg Gln Gln Lys Phe Tyr Leu Glu Thr Gln Ala
    850                 855                 860

Gly Lys Leu Glu Ala Gln Asn Arg Lys Leu Glu Glu Gln Leu Glu Lys
865                 870                 875                 880
```

```
Ile Ser His Gln Asp His Ser Asp Lys Ser Arg Leu Leu Glu Leu Glu
                885                 890                 895

Thr Arg Leu Arg Glu Val Ser Leu Glu His Glu Glu Gln Lys Leu Glu
            900                 905                 910

Leu Lys Arg Gln Leu Thr Glu Leu Gln Leu Ser Leu Gln Glu Arg Glu
        915                 920                 925

Ser Gln Leu Thr Ala Leu Gln Ala Ala Arg Ala Ala Leu Glu Ser Gln
    930                 935                 940

Leu Arg Gln Ala Lys Thr Glu Leu Glu Glu Thr Thr Ala Glu Ala Glu
945                 950                 955                 960

Glu Glu Ile Gln Ala Leu Thr Ala His Arg Asp Glu Ile Gln Arg Lys
                965                 970                 975

Phe Asp Ala Leu Arg Asn Ser Cys Thr Val Ile Thr Asp Leu Glu Glu
            980                 985                 990

Gln Leu Asn Gln Leu Thr Glu Asp  Asn Ala Glu Leu Asn  Asn Gln Asn
        995                 1000                1005

Phe Tyr  Leu Ser Lys Gln Leu  Asp Glu Ala Ser Gly  Ala Asn Asp
    1010                1015                1020

Glu Ile  Val Gln Leu Arg Ser  Glu Val Asp His Leu  Arg Arg Glu
    1025                1030                1035
```

```
Ile Thr  Glu Arg Glu Met Gln  Leu Thr Ser Gln Lys  Gln Thr Met
    1040                 1045                 1050

Glu Ala  Leu Lys Thr Thr Cys  Thr Met Leu Glu Glu  Gln Val Leu
    1055                 1060                 1065

Asp Leu  Glu Ala Leu Asn Asp  Glu Leu Leu Glu Lys  Glu Arg Gln
    1070                 1075                 1080

Trp Glu  Ala Trp Arg Ser Val  Leu Gly Asp Glu Lys  Ser Gln Phe
    1085                 1090                 1095

Glu Cys  Arg Val Arg Glu Leu  Gln Arg Met Leu Asp  Thr Glu Lys
    1100                 1105                 1110

Gln Ser  Arg Ala Arg Ala Asp  Gln Arg Ile Thr Glu  Ser Arg Gln
    1115                 1120                 1125

Val Val  Glu Leu Ala Val Lys  Glu His Lys Ala Glu  Ile Leu Ala
    1130                 1135                 1140

Leu Gln  Gln Ala Leu Lys Glu  Gln Lys Leu Lys Ala  Glu Ser Leu
    1145                 1150                 1155

Ser Asp  Lys Leu Asn Asp Leu  Glu Lys Lys His Ala  Met Leu Glu
    1160                 1165                 1170

Met Asn  Ala Arg Ser Leu Gln  Gln Lys Leu Glu Thr  Glu Arg Glu
    1175                 1180                 1185
```

```
Leu Lys  Gln Arg Leu Leu Glu  Glu Gln Ala Lys Leu  Gln Gln Gln
    1190                 1195                 1200

Met Asp  Leu Gln Lys Asn His  Ile Phe Arg Leu Thr  Gln Gly Leu
    1205                 1210                 1215

Gln Glu  Ala Leu Asp Arg Ala  Asp Leu Leu Lys Thr  Glu Arg Ser
    1220                 1225                 1230

Asp Leu  Glu Tyr Gln Leu Glu  Asn Ile Gln Val Leu  Tyr Ser His
    1235                 1240                 1245

Glu Lys  Val Lys Met Glu Gly  Thr Ile Ser Gln Gln  Thr Lys Leu
    1250                 1255                 1260

Ile Asp  Phe Leu Gln Ala Lys  Met Asp Gln Pro Ala  Lys Lys Lys
    1265                 1270                 1275

Lys Val  Pro Leu Gln Tyr Asn  Glu Leu Lys Leu Ala  Leu Glu Lys
    1280                 1285                 1290

Glu Lys  Ala Arg Cys Ala Glu  Leu Glu Glu Ala Leu  Gln Lys Thr
    1295                 1300                 1305

Arg Ile  Glu Leu Arg Ser Ala  Arg Glu Glu Ala Ala  His Arg Lys
    1310                 1315                 1320

Ala Thr  Asp His Pro His Pro  Ser Thr Pro Ala Thr  Ala Arg Gln
    1325                 1330                 1335
```

```
Gln Ile  Ala Met Ser Ala Ile  Val Arg Ser Pro Glu  His Gln Pro
    1340                 1345                 1350

Ser Ala  Met Ser Leu Leu Ala  Pro Pro Ser Ser Arg  Arg Lys Glu
    1355                 1360                 1365

Ser Ser  Thr Pro Glu Glu Phe  Ser Arg Arg Leu Lys  Glu Arg Met
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His His  Asn Ile Pro His Arg  Phe Asn Val Gly Leu  Asn Met Arg
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Ala Thr  Lys Cys Ala Val Cys  Leu Asp Thr Val His  Phe Gly Arg
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Gln Ala  Ser Lys Cys Leu Glu  Cys Gln Val Met Cys  His Pro Lys
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Cys Ser  Thr Cys Leu Pro Ala  Thr Cys Gly Leu Pro  Ala Glu Tyr
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Ala Thr  His Phe Thr Glu Ala  Phe Cys Arg Asp Lys  Met Asn Ser
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Pro Gly  Leu Gln Ser Lys Glu  Pro Gly Ser Ser Leu  His Leu Glu
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Gly Trp  Met Lys Val Pro Arg  Asn Asn Lys Arg Gly  Gln Gln Gly
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Trp Asp  Arg Lys Tyr Ile Val  Leu Glu Gly Ser Lys  Val Leu Ile
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Tyr Asp  Asn Glu Ala Arg Glu  Ala Gly Gln Arg Pro  Val Glu Glu
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Phe Glu  Leu Cys Leu Pro Asp  Gly Asp Val Ser Ile  His Gly Ala
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Val Gly  Ala Ser Glu Leu Ala  Asn Thr Ala Lys Ala  Asp Val Pro
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Tyr Ile  Leu Lys Met Glu Ser  His Pro His Thr Thr  Cys Trp Pro
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Gly Arg  Thr Leu Tyr Leu Leu  Ala Pro Ser Phe Pro  Asp Lys Gln
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Arg Trp  Val Thr Ala Leu Glu  Ser Val Val Ala Gly  Gly Arg Val
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Ser Arg  Glu Lys Ala Glu Ala  Asp Ala Lys Leu Leu  Gly Asn Ser
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Leu Leu  Lys Leu Glu Gly Asp  Asp Arg Leu Asp Met  Asn Cys Thr
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Leu Pro  Phe Ser Asp Gln Val  Val Leu Val Gly Thr  Glu Glu Gly
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Lys Leu  Leu Met Ile Ala Gly  Glu Glu Arg Ala Leu  Cys Leu Val
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Asp Val  Lys Lys Val Lys Gln  Ser Leu Ala Gln Ser  His Leu Pro
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Ala Gln  Pro Asp Val Ser Pro  Asn Ile Phe Glu Ala  Val Lys Gly
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Cys His  Leu Phe Ala Ala Gly  Lys Ile Glu Asn Ser  Leu Cys Ile
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Cys Ala  Ala Met Pro Ser Lys  Val Val Ile Leu Arg  Tyr Asn Asp
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Asn Leu  Ser Lys Tyr Cys Ile  Arg Lys Glu Ile Glu  Thr Ser Glu
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Pro Cys  Ser Cys Ile His Phe  Thr Asn Tyr Ser Ile  Leu Ile Gly
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Glu Phe  Leu Asp Lys Asn Asp  His Ser Leu Ala Pro  Ala Val Phe
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Ala Ser  Ser Ser Asn Ser Phe  Pro Val Ser Ile Val  Gln Ala Asn
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Ser Ala  Gly Gln Arg Glu Glu  Tyr Leu Leu Cys Phe  His Glu Phe
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Gly Val  Phe Val Asp Ser Tyr  Gly Arg Arg Ser Arg  Thr Asp Asp
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Leu Lys  Trp Ser Arg Leu Pro  Leu Ala Phe Ala Tyr  Arg Glu Pro
    1850                 1855                 1860

Tyr Leu  Phe Val Thr His Phe  Asn Ser Leu Glu Val  Ile Glu Ile
    1865                 1870                 1875

Gln Ala  Arg Ser Ser Leu Gly  Ser Pro Ala Arg Ala  Tyr Leu Glu
    1880                 1885                 1890

Ile Pro  Asn Pro Arg Tyr Leu  Gly Pro Ala Ile Ser  Ser Gly Ala
    1895                 1900                 1905

Ile Tyr  Leu Ala Ser Ser Tyr  Gln Asp Lys Leu Arg  Val Ile Cys
    1910                 1915                 1920

Cys Lys  Gly Asn Leu Val Lys  Glu Ser Gly Thr Glu  Gln His Arg
    1925                 1930                 1935
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Val Pro Ser Thr Ser Arg Ser Ser Pro Asn Lys Arg Gly Pro Pro
1940 1945 1950

Thr Tyr Asn Glu His Ile Thr Lys Arg Val Ala Ser Ser Pro Ala
1955 1960 1965

Pro Pro Glu Gly Pro Ser His Pro Arg Glu Pro Ser Thr Pro His
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Arg Tyr Arg Asp Arg Glu Gly Arg Thr Glu Leu Arg Arg Asp Lys
1985 1990 1995

Ser Pro Gly Arg Pro Leu Glu Arg Glu Lys Ser Pro Gly Arg Met
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Leu Ser Thr Arg Arg Glu Arg Ser Pro Gly Arg Leu Phe Glu Asp
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Glu Ser Gln Leu Thr Ala Leu Gln Ala Ala Arg Ala Ala Leu Glu Ser
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Gln Leu Arg Gln Ala Lys Thr Glu Leu Glu Glu Thr Thr Ala Glu Ala
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Glu Glu Glu Ile Gln Ala Leu Thr Ala His Arg Asp Glu Ile Gln Arg
        195                 200                 205

Lys Phe Asp Ala Leu Arg Asn Ser Cys Thr Val Ile Thr Asp Leu Glu
    210                 215                 220

Glu Gln Leu Asn Gln Leu Thr Glu Asp Asn Ala Glu Leu Asn Asn Gln
225                 230                 235                 240

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Glu Ile Val Gln Leu Arg Ser Glu Val Asp His Leu Arg Arg Glu Ile
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305                 310                 315                 320

Arg Ser Val Leu Gly Asp Glu Lys Ser Gln Phe Glu Cys Arg Val Arg
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Glu Leu Gln Arg Met Leu Asp Thr Glu Lys Gln Ser Arg Ala Arg Ala
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Asp Gln Arg Ile Thr Glu Ser Arg Gln Val Val Glu Leu Ala Val Lys
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Glu His Lys Ala Glu Ile Leu Ala Leu Gln Gln Ala Leu Lys Glu Gln
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Lys His Ala Met Leu Glu Met Asn Ala Arg Ser Leu Gln Gln Lys Leu
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Ile Asp Phe Leu Gln Ala Lys Met Asp Gln Pro Ala Lys Lys Lys Lys
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Leu Arg Ser Ala Arg Glu Glu Ala Ala His Arg Lys Ala Thr Asp His
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                565                 570                 575

Ala Ile Val Arg Ser Pro Glu His Gln Pro Ser Ala Met Ser Leu Leu
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Met Cys His Pro Lys Cys Ser Thr Cys Leu Pro Ala Thr Cys Gly Leu
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Pro Ala Glu Tyr Ala Thr His Phe Thr Glu Ala Phe Cys Arg Asp Lys
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Met Asn Ser Pro Gly Leu Gln Thr Lys Glu Pro Ser Ser Ser Leu His
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Leu Glu Gly Trp Met Lys Val Pro Arg Asn Asn Lys Arg Gly Gln Gln
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Gly Trp Asp Arg Lys Tyr Ile Val Leu Glu Gly Ser Lys Val Leu Ile
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Tyr Asp Asn Glu Ala Arg Glu Ala Gly Gln Arg Pro Val Glu Glu Phe
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Glu Leu Cys Leu Pro Asp Gly Asp Val Ser Ile His Gly Ala Val Gly
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Ala Ser Glu Leu Ala Asn Thr Ala Lys Ala Asp Val Pro Tyr Ile Leu
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Lys Met Glu Ser His Pro His Thr Thr Cys Trp Pro Gly Arg Thr Leu
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Tyr Leu Leu Ala Pro Ser Phe Pro Asp Lys Gln Arg Trp Val Thr Ala
                805                 810                 815

Leu Glu Ser Val Val Ala Gly Gly Arg Val Ser Arg Glu Lys Ala Glu
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Ala Asp Ala Lys Leu Leu Gly Asn Ser Leu Leu Lys Leu Glu Gly Asp
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Asp Arg Leu Asp Met Asn Cys Thr Leu Pro Phe Ser Asp Gln Val Val
    850                 855                 860

Leu Val Gly Thr Glu Glu Gly Leu Tyr Ala Leu Asn Val Leu Lys Asn
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Ser Leu Thr His Val Pro Gly Ile Gly Ala Val Phe Gln Ile Tyr Ile
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Ile Lys Asp Leu Glu Lys Leu Leu Met Ile Ala Gly Glu Glu Arg Ala
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Leu Cys Leu Val Asp Val Lys Lys Val Lys Gln Ser Leu Ala Gln Ser
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His Leu Pro Ala Gln Pro Asp Ile Ser Pro Asn Ile Phe Glu Ala Val
    930                 935                 940

Lys Gly Cys His Leu Phe Gly Ala Gly Lys Ile Glu Asn Gly Leu Cys
945                 950                 955                 960

Ile Cys Ala Ala Met Pro Ser Lys Val Val Ile Leu Arg Tyr Asn Glu
                965                 970                 975

Asn Leu Ser Lys Tyr Cys Ile Arg Lys Glu Ile Glu Thr Ser Glu Pro
            980                 985                 990

Cys Ser Cys Ile His Phe Thr Asn  Tyr Ser Ile Leu Ile  Gly Thr Asn
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Lys Phe  Tyr Glu Ile Asp Met  Lys Gln Tyr Thr Leu  Glu Glu Phe
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Leu Asp  Lys Asn Asp His Ser  Leu Ala Pro Ala Val  Phe Ala Ala
    1025                1030                1035

Ser Ser  Asn Ser Phe Pro Val  Ser Ile Val Gln Val  Asn Ser Ala
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Gly Gln  Arg Glu Glu Tyr Leu  Leu Cys Phe His Glu  Phe Gly Val
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Phe Val  Asp Ser Tyr Gly Arg  Arg Ser Arg Thr Asp  Asp Leu Lys
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Trp Ser  Arg Leu Pro Leu Ala  Phe Ala Tyr Arg Glu  Pro Tyr Leu
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Phe Val  Thr His Phe Asn Ser  Leu Glu Val Ile Glu  Ile Gln Ala
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Arg Ser  Ser Ala Gly Thr Pro  Ala Arg Ala Tyr Leu  Asp Ile Pro
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Asn Pro  Arg Tyr Leu Gly Pro  Ala Ile Ser Ser Gly  Ala Ile Tyr
    1130                 1135                 1140

Leu Ala  Ser Ser Tyr Gln Asp  Lys Leu Arg Val Ile  Cys Cys Lys
    1145                 1150                 1155

Gly Asn  Leu Val Lys Glu Ser  Gly Thr Glu His His  Arg Gly Pro
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Ser Thr  Ser Arg Ser Ser Pro  Asn Lys Arg Gly Pro  Pro Thr Tyr
    1175                 1180                 1185

Asn Glu  His Ile Thr Lys Arg  Val Ala Ser Ser Pro  Ala Pro Pro
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Glu Gly  Pro Ser His Pro Arg  Glu Pro Ser Thr Pro  His Arg Tyr
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His Gln Glu Leu Gly Ala Ser His Leu Ala Gln Asp Lys Tyr Val Ala
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Arg Leu Gln Arg Asp Asp Phe Glu Ile Leu Lys Val Ile Gly Arg Gly
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Ala Phe Ser Glu Val Ala Val Val Lys Met Lys Gln Thr Gly Gln Val
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Tyr Ala Met Lys Ile Met Asn Lys Trp Asp Met Leu Lys Arg Gly Glu
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Val Ser Cys Phe Arg Glu Glu Arg Asp Val Leu Val Lys Gly Asp Arg
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Arg Trp Ile Thr Gln Leu His Phe Ala Phe Gln Asp Glu Asn Tyr Leu
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Tyr Leu Val Met Glu Tyr Tyr Val Gly Gly Asp Leu Leu Thr Leu Leu
145                 150                 155                 160

Ser Lys Phe Gly Glu Arg Ile Pro Ala Glu Met Ala Arg Phe Tyr Leu
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Ala Glu Ile Val Met Ala Ile Asp Ser Val His Arg Leu Gly Tyr Val
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His Arg Asp Ile Lys Pro Asp Asn Ile Leu Leu Asp Arg Cys Gly His
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Ile Arg Leu Ala Asp Phe Gly Ser Cys Leu Lys Leu Gln Pro Asp Gly
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Met Val Arg Ser Leu Val Ala Val Gly Thr Pro Asp Tyr Leu Ser Pro
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Glu Ile Leu Gln Ala Val Gly Gly Gly Pro Gly Ala Gly Ser Tyr Gly
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Pro Glu Cys Asp Trp Trp Ala Leu Gly Val Phe Ala Tyr Glu Met Phe
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Tyr Gly Gln Thr Pro Phe Tyr Ala Asp Ser Thr Ala Glu Thr Tyr Ala
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Lys Ile Val His Tyr Arg Glu His Leu Ser Leu Pro Leu Ala Asp Thr
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Val Val Pro Glu Glu Ala Gln Asp Leu Ile Arg Gly Leu Leu Cys Pro
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Ala Glu Ile Arg Leu Gly Arg Gly Gly Ala Gly Asp Phe Gln Lys His
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Pro Phe Phe Phe Gly Leu Asp Trp Glu Gly Leu Arg Asp Ser Val Pro
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Pro Phe Thr Pro Asp Phe Glu Gly Ala Thr Asp Thr Cys Asn Phe Asp
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Val Val Glu Asp Arg Leu Thr Ala Met Val Ser Gly Gly Gly Glu Thr
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Leu Ser Asp Met Gln Glu Asp Met Pro Leu Gly Val Arg Leu Pro Phe
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Val Gly Tyr Ser Tyr Cys Cys Met Ala Phe Arg Asp Asn Gln Val Pro
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Asp Pro Thr Pro Met Glu Leu Glu Ala Leu Gln Leu Pro Val Ser Asp
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Ala Glu Glu Ala Asp Leu Val Ala Val Pro Ala Pro Val Ala Glu Ala
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Glu Thr Thr Val Thr Leu Gln Gln Leu Gln Glu Ala Leu Glu Glu Glu
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Val Leu Thr Arg Gln Ser Leu Ser Arg Glu Leu Glu Ala Ile Arg Thr
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Ala Asn Gln Asn Phe Ser Ser Gln Leu Gln Glu Ala Glu Val Arg Asn
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Arg Asp Leu Glu Ala His Val Arg Gln Leu Gln Glu Arg Met Glu Met
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Leu Gln Ala Pro Gly Ala Ala Ala Ile Thr Gly Val Pro Ser Pro Arg
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Ala Thr Asp Pro Pro Ser His Leu Asp Gly Pro Pro Ala Val Ala Val
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Gly Gln Cys Pro Leu Val Gly Pro Gly Pro Met His Arg Arg His Leu
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Leu Leu Pro Ala Arg Ile Pro Arg Pro Gly Leu Ser Glu Ala Arg Cys
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cccgggaggc tgtttgaaga cagcagcagg ggccggctgc ctgcgggagc cgtgaggacc 6120

ccgctgtccc aggtgaacaa ggtgaggcag cattcc 6156

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/07156

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/435 C12N15/52 C12N5/10 C12N9/00 C12Q1/68
G01N33/53 G01N33/573 A61P9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N C12Q G01N A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, SEQUENCE SEARCH, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 38503 A (PLOWMAN GREGORY D ;CLARY DOUGLAS (US); SUGEN INC (US); WHYTE DAVID) 31 May 2001 (2001-05-31) SEQ ID No 1 tables 1,3 ---	1-12, 15-17
X	BARTON G J: "PROTEIN SEQUENCE ALIGMENT AND DATABASE SCANNING" PROTEIN STRUCTURE PREDICTION. A PRACTICAL APPROACH, XX, XX, 1996, pages 31-63, XP000829540 the whole document --- -/--	1-12, 15-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 December 2002	11/12/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Keller, Y

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GEORGE D G ET AL: "CURRENT METHODS IN SEQUENCE COMPARISON AND ANALYSIS" MACROMOLECULAR SEQUENCING AND SYNTHESIS SELECTED METHODS AND APPLICATIONS, XX, XX, 1988, pages 127-149, XP000829541 the whole document	1-12, 15-17
Y	MADAULE PASCAL ET AL: "A novel partner for the GTP-bound forms of rho and rac" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 377, no. 2, 1995, pages 243-248, XP002200178 ISSN: 0014-5793 the whole document	1-12, 15-17
Y	DI CUNTO FERDINANDO ET AL: "Citron Rho-interacting kinase, a novel tissue-specific Ser/Thr kinase encompassing the Rho-Rac-binding protein citron" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 273, no. 45, 6 November 1998 (1998-11-06), pages 29706-29711, XP002170360 ISSN: 0021-9258 the whole document	1-12, 15-17
Y	NAGASE ET AL: "PREDICTION OF THE CODING SEQUENCE OF UNIDENTIFIED HUMAN GENES. XIII. THE COMPLETE SEQUENCE OF 100 NEW CDNA CLONES FROM BRAIN WHICH CODE FOR LARGE PROTEINS IN VITRO" DNA RESEARCH, UNIVERSAL ACADEMY PRESS, JP, vol. 6, 1999, pages 63-70, XP000952912 ISSN: 1340-2838 the whole document	1-12, 15-17

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 13, 14 and 15-17 partially

Present claims 13, 14 and 15-17 partially relate to an extremely large number of possible compounds/methods. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/products/apparatus/methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/07156

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 13, 14 and 15-17 partially
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/07156

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0138503 A	31-05-2001	AU 1926001 A	04-06-2001
		EP 1240194 A2	18-09-2002
		WO 0138503 A2	31-05-2001